THE FIGHT AGAINST DISEASE

CHEMOTHERAPY, ANTIBIOTICS AND IMMUNITY

Plate 1. *Frontispiece*: Sir Alexander Fleming examining cultures with Dr. Selman A. Waksman in the latter's laboratory, University of Rutgers, April 1951.

The Fight Against Disease

Chemotherapy, Antibiotics and Immunity

R. KALYANASUNDARAM

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Foreword

THE USEFULNESS of science as a subject of study has indeed been recognised during the last few decades. But now, more than ever, when the country has launched tremendous economic and social welfare programmes which hinge upon the application of the latest advances in science and technology, it has become essential that every citizen should have as much knowledge of science as possible. While science is now being made a compulsory subject at the school level and textbooks based on new concepts of science teaching are being introduced, a textbook can only impart knowledge within the limits of the school syllabus. Therefore, in an attempt to make easily accessible to young minds additional and more comprehensive information in the various areas of science, the National Council is preparing and bringing out a series of supplementary reading materials on science. The present book is one of this series, and I hope that it will be found useful not only by students but by teachers and others as well.

RAIS AHMED
Director
National Council of Educational
Research and Training

New Delhi
18 February 1976

Introduction

It is seldom realised that in the fight against disease the role of the biologist has been of considerable importance comparable to those great pioneers in other disciplines of science. The progress of the medical science has been closely related in every stage to the various facets of human civilization itself and the cultural developments which impinge on the minds of human society.

The long road of healing human ailments—from faith cure to medical therapy—has been no avenue of sylvan beauty, but on the other hand, it has been a rugged path with many hazards. That some measure of success has flown out along this long and arduous path, is a tribute by itself to the human ingenuity of the many pioneers who chose this path, well aware of the challenge ahead of them. The treatment of diseases by the use of either naturally occurring chemicals or those synthesized in laboratories has mostly been a story of the last 100 years or so. Healing through the use of diverse chemical agents was not an unknown art, but a thorough understanding of the basis of chemotherapy is only of recent origin. Causes of maladies were known. Nevertheless, the causative agents eluded the eyes of several of the giants and pioneers. Paracelsus, the father of chemotherapy, initiated the use of mercury in chemotherapy. But not till Ehrlich's 'Salvarsan' was triumphantly put in the market could one claim that chemotherapy was on a road all its own.

The discovery of the bacteria and their role in diseases and the following attempts of Ehrlich, Domagk and others to-day read like the pages of a thriller. Thrilling it has been. The tasks of these pioneers culminated in Fleming discovering penicillin, Waksman giving new hope to tubercular patients with streptomycin and still many others who have collectively given mankind new hopes in combating otherwise fatal ailments of microbial origin. With these discoveries also arose the problem of the versatile organisms developing resistance to these new drugs and antibiotics. So the battle is one of wits, and who is the winner or the vanquished has not been answered. New concepts of antigens and antibodies interacting with each other in the human system have emerged introducing a new plane of thought in therapeutics. Can disease be prevented ? Are all our social problems based on disease capable of final solution ? Is human endeavour the final victor in the battle against disease ? Who can say ?

It was a bold venture on our part to have asked a person outside the field of medical science, like Dr. Kalyanasundaram, to attempt to write a small booklet on 'The Fight Against Disease' covering fields of chemotherapy, antibiotics and immunity. Dr. Kalyanasundaram represents a generation of fresh thinking that has emerged of late in this country, with a background of training in this country and abroad. He has been interested in important aspects of immunology and antigen-antibody relationships in host-parasite interactions. It was only natural for us to think of him as the author of the present booklet and he has justified our confidence in him. We only hope that the doors of knowledge these young men have opened, to let the younger generations in our schools and colleges to have a glimpse of the vast vistas ahead, will open wider. If we attain this end, we shall be more than satisfied.

University Botany Laboratory
Madras

T. S. SADASIVAN

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7. Figures of original plates of Fleming with the mould contaminant, a gutter plate, prepared by Fleming's method, assay of penicillin, Heatly's assay method and morphological changes in bacteria caused by penicillin, a close look at colonies of *Penicillium notatum*—courtesy Professor E. B. Chain, FRS, NL and Endeavour, London.

8. Picture of Edward Jenner vaccinating a child—courtesy Bettman Archives and Cambridge University Press, London.

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1

The Early Groping

THE AGE OF THE EARTH has been estimated to be about five billion (million × million) years. Man is supposed to have arrived rather late in the earth's history—about a million years ago. We have recorded history of man only for the past 4000 to 5000 years. But if one goes through these records, however scanty they are, it is clear that man has always been at battle with his environment, with floods and drought, famine and warring neighbours. More than all these, mankind has been suffering from ravages brought about by pestilence and diseases. It was only a century ago that the enemy was identified and a systematic treatment of disease by chemicals started; this is known as chemotherapy. It is essentially a contribution of the scientists of the nineteenth and twentieth centuries. But it is impossible to visualise its full impact without understanding the historical perspective—the men and their fighting spirits through the ages which cumulatively helped in these discoveries.

*

The slow progress of medical science towards an understanding of the causes of diseases, and thus towards the proper scientific attack against them, was chiefly due to a complete lack of factual knowledge of the body. For generations the dissection of the dead was a taboo and even today it is considered to be against sentiments.

For thousands of years all over the world the approach to disease was based on religious concepts and on a conviction that the gods, for some reason known only to them, created demonic ailments of the human body. Each disease was attributed to some evil spirit. The religious attitude towards diseases persists even today amongst our people—the attitude of our people to an outbreak of small-pox, for example —and this attitude had stood in the way of development of chemotherapy.

We have faith-healers still with us. They may probably help individual patients suffering from functional disorders, nervous disorders etc., but throughout the ages they have stood in the way of scientific approach to diseases caused by germs.

The first real objective study of the symptom of disease, a study which ultimately led to a knowledge of the cause and to the chemical attack against it, was made in our country as early as the Post-Vedic period (500 B.C.—A.D. 600). The period prior to this, namely, Pre-Vedic (10000 B.C.—1500 B.C.) was dominated by the concept of disease as due to demons and spirit. In the Vedic period (1500 B.C.—500 B.C.) the *Atharva Veda* enumerates several causes of diseases. The concept of contagion was vaguely present and is a remarkable observation for that age. During this period rudimentary ideas of physiological processes, like digestion of food were known. But in the history of man, one can say that the Post-Vedic age in India may be identified as the period of peak glory for Indian Medicine and is known as the age of *Ayurveda* (knowledge of life). Three physiological factors like *vayu* (wind), *pitta* (bile) and *kapha* (phlegm) were considered responsible for the condition of the body in health and disease. Susruta who lived around 500 B.C. was considered to have been much ahead of his time in propounding these principles, himself being a great surgeon. However, this great advance in the rational approach and treatment of the diseases was not pursued in India by the men of the succeeding generation to lead on to certain logical discoveries. A sense of stagnation could be noticed in the Indian scene. The advent of modern medicine due to the recent advances made in the field of science and technology is essentially a product of the civilization of the West—West, which, however, was steeped in ignorance and believed in the demonic origin of disease when Susruta and others were making significant contribution to the knowledge of medicine in India.

Hippocrates of Greece founded the first school of medicine in Europe and he tried to emancipate medicine from superstition. He lived during the great age of Pericles, Socrates and Plato, 150 years after Susruta. It was Hippocrates' honesty, and his scientific approach, which, quite apart from his ethical standards, have caused him to be called the Founder of Modern Medicine. He discarded the demonic theory of disease. This was a revolution in human thought. Yet, Hippocrates had no idea of the fact that certain diseases were contagious. However, a contemporary historian of Hippocrates, Thucydides, did observe thus about a dreaded disease: "Appalling was the rapidity with which men caught the infection, dying like sheep if they attended to one another, and this was the principal cause of mortality. No one was ever attacked a second time, or at least not with a fatal result." Possibly he was referring to plague.

In the first century B.C., Marcus Terentius Varro came very near the truth and vaguely foretold the discovery of bacteria. He wrote, "Small creatures, invisible to eye, fill the atmosphere in many localities and with the air breathed through the nose and mouth penetrate into the human body, thereby causing dangerous diseases."

In the second century A.D., we have to conjure up the name of Galen—the greatest anatomist and experimental physiologist of the age. Galen built a well-developed system of medicine which was to form the basis for the treatment of all diseases. He had very little to say about the contagious nature of diseases. Galen believed that the human body was made up of four elements—fire, air, water and earth. These had four basic qualities—hot, dry, moist and cold. To maintain health, each of the qualities had to be maintained in a natural proportion; and disease arose when the balance got disturbed. For example, disease due to excessive heat is to be treated with cooling remedies. This concept is very similar to the Ayurvedic concept of the Post-Vedic India (500 B.C.—A.D. 600). It is of interest to note the independent, yet similar, thought that was then current. Cool as a cucumber and hot as pepper are Galenic expressions which one hears even today.

Another ten centuries of groping lead on to the age of the alchemists in the twelfth century A.D. The term alchemy is derived from the Arabic word 'khem' which is the native name for Egypt and 'al' is an Arabic prefix.

Alchemy was more of a secret art than a science. In their efforts to transmute base metals into gold or silver, the alchemists were unconsciously acting as forerunners of modern chemists, as they were the first to carry on experiments in laboratories. In the same blind manner, some alchemists by accident also stumbled unknowingly on the path which finally led to the chemical attack against disease. They started making all sorts of concoctions and were looking for a supreme remedy for all diseases, the 'Elixir'. Many alchemists fancied themselves as medical discoverers and majority of the physicians of that time meddled in alchemy.

Raymond Lully, one of the famous alchemists of the fourteenth century, alleged that he had secretly transmuted huge quantities of other metals into gold, yet, side by side with this false claim, he made a real contribution when he concocted alcoholic preparations of drugs, which today go under the name of 'tinctures'.

Great universities were founded in Europe around this time and yet the real cause of disease was not studied. The acceptance of the knowledge that contagion and infection exist was not however general until the terrible pandemic of bubonic plague in 1347 which swept across Europe from Asia and Constantinople. It is estimated that 25 per cent of the human race perished in this epidemic. People were really clamouring to know the cause of such a serious tragedy and finally lay men and physicians started to appreciate that contagion was a real fact.

So many revolutionary changes occurred in science, geography, religion and art in the second half of the fifteenth century that medicine too, was inevitably affected. Printing had been invented and the earliest printed medical books were available in 1457. Leonardo da Vinci was experimenting with flying machines; Nicholas Copernicus taught that the sun, and not the earth, is the centre of the planetary system; Columbus proved by his discovery of the new world that the earth is round and not flat; Vasco da Gama found the ocean route to India from Europe. By these discoveries the mental horizon of people had widened and a new spirit of questioning and revolt started spreading all over the world similar to what one witnesses today after the landing of the first man on the moon. It was not, therefore, surprising that the two outstanding figures of the fifteenth and sixteenth century medicine should have accomplished much in curing certain diseases—Fracastorius of Italy and Paracelsus of Switzerland. While Fracastorius could decipher the contagious nature of syphilis (infection of the genital organs) Paracelsus made a very careful study of the effect of chemicals on diseases. Paracelsus has been often called the "Father of Chemotherapy". He was concerned with the occupational diseases of miners and he was not only concerned with pulmonary diseases of miners but also made a specialised study of certain ailments caused by mercury poisoning. He was the first to take chemical factors into account and he argued that if some of these chemicals were poisonous some of them may also have a salubrious effect on the human body. He was concerned with mercury, the chemical, which centuries later, became the basis of Professor Ehrlich's famous salvarsan, *acknowledged as the first scientifically discovered chemotherapeutant.* He investigated amalgam of mercury and amalgam of copper. He studied the various uses of alum; he analysed bismuth. He discovered zinc and called it as half-metal. He discovered calomel and flowers of sulphur and worked on many other distillations which he added to the pharmacopoeia. He was the first medical practitioner to give chemical substances internally as well as salves. He learned in the mines, the use of compounds of antimony and mercury, which he employed for curing syphilis. He also began to understand the specificity of diseases and that such diseases may be cured by specific drugs, which he called *arcana.* Without microscopes and sufficiently developed sciences of chemistry and pathology, no man could have done as well as Paracelsus did.

2

The Early Fight Against Disease

DURING THE SEVENTEENTH CENTURY there were great developments in the spheres of human knowledge other than medicine and chemistry. This did have its impact on future developments in the field of chemotherapy. It was the age of physics and mathematics, the age of giants like Galileo, Newton, Kepler, Locke, Bacon and Spinoza; men who contributed to the development of experimental science. No less important was the construction of the tiny microscope by the Dutch cloth merchant, Antonie von Leeuwenhoek, and his observation of the tiny animals; suddenly, the until-then-unknown bacteria were discovered.

Leeuwenhoek's sighting of the bacteria, as well as the outstanding contribution of the discovery of the amazing medicinal uses of certain natural products in the 17th century appear to have shortened the otherwise long path that led to the successful accomplishment of chemotherapy in the 19th century. Chief among the natural products discovered was the cinchona bark, which, in its modern form, quinine, has become of vital importance in the treatment of that protozoan infection still known as malaria. The name 'mal-aria' (bad air) was given to the disease in the late seventeenth century by an Italian physician, Torti, who was one of the pioneers in the use of cinchona bark. The remedy was discovered earlier than the cause of the disease, the protozoan parasites were discovered only in 1880 when Alphones Laveran, a French army surgeon, recognised them in the red blood cells of malarial patients in a hospital in Algeria.

The bark of cinchona tree was first used in the primeval forests of Peru in South America. The natives there had found out that, when prepared into a bitter brew, it cured them of a fever. They made this discovery by sheer experience, just as they had learned that when they climbed the steep passes of the Andes mountains they felt less exhausted by chewing leaves of cocoa. The natives have made two revolutionary discoveries—quinine and the narcotic cocaine.

A Jesuit priest, who had worked among the native Incas of Peru, was suffering from a serious illness caused by intermittent fever. He was cured by the medicine man of the native village who had administered him a strong brew. After much persuasion the Jesuit priest learned the source of the medicine and he returned with a bundle of the miraculous bark from the interior of the forest to the town of Loxa. At that time, Ana, the countess of Chincon, fell ill in Peru. Her physician was desparately looking for the legendary barks. The Jesuit father could use the bark and cure her of her illness. The bark powder was immediately sent to Europe where it was known by many names: Countess powder, Peruvian bark etc. The tree in any case was named after the countess for all time by Linnaeus himself and is today called the cinchona tree.

It is the work on the cinchona bark two centuries later that opened the door to one of the greatest discoveries of the nineteenth century. In 1850, an 18-year-old British scientist, W. H. Perkin, tried to produce artificial quinine. In the course of his experiments he stumbled on the dye aniline purple. This black preparation, aniline purple, belonging to a family of coal-tar dyes, suddenly became very important. We will see later how Robert Koch used aniline dyes to stain the bacteria; his student Paul Ehrlich got his idea of chemotherapy while hobnobbing with the dyes and bacteria. Medically also, the aniline dyes became important: we will see later on that the sulphanilamide group of drugs—prontosil for example, the first ever antibacterial drug scientifically discovered around 1930,—belongs to the chemical group of coal-tar dye like aniline purple. Perkin's contribution, therefore, led directly to these chemotherapeutic remedies. This is an excellent example of independent and disconnected discoveries through the ages, cumulatively yielding in the long run, to outstanding contribution in science. Science is not for individualists!

The long-drawn-out battle against many diseases could be successfully terminated by the end of the nineteenth century, thanks to the epoch-making discoveries of Louis Pasteur of France and Robert Koch of Germany. Rarely is it given in the life of an individual to make a single discovery. Rarer ill is it given to any individual to make seven astounding disoveries: Louis Pasteur had this distinction. One of his iscoveries was the 'germ theory' of disease, that is, specific erms cause specific diseases. Thus with the enemy identified 1857) chemical treatment of diseases on a scientific basis sudenly appeared possible.

Robert Koch, a contemporary of Louis Pasteur, not only onfirmed the germ theory of disease by identifying it but he amed the enemy. Using the newly discovered aniline dyes e could stain and observe the bacteria under the microscope which he showed were rod-shaped. His pioneering work was with bacilli that cause anthrax in cattle. It was Koch who discovered the causal agent of tuberculosis. He demonstrated that bacteria cause infectious diseases. The rigorous method he enunciated to prove this is even today used by bacteriologists world over, known as "Koch's postulate" to establish the cause of any disease.

When Robert Koch was wiping out centuries of man's ignorance about the origin of diseases, a student of his, Paul Ehrlich, was starting the scientific fight against bacteria using chemicals, and finally he discovered the first ever chemotherapeutant, a forerunner of many new drugs used today against bacterial, fungal and protozoan infections of man.

3

Chemotherapy

EHRLICH was one of Koch's assistants and the only son of an innkeeper at Strehlen, a small town near Breslau in Germany. The word 'Chemotherapy' was coined by him. He completed his High School at Breslau and continued his medical studies at the University of Breslau. He was not considered to be a brilliant student by his teachers. Ehrlich was introduced to Robert Koch when he came to Breslau, as an undergraduate who was always playing with chemistry. He was all the time hobnobbing with the staining properties of coal-tar dyes, on animal tissue. The coal-tar dyes, it may be mentioned here, were discovered a few years earlier, in 1850, by Perkin when he was attempting to synthesize quinine. Ehrlich found that the dye methylene blue when injected into a rabbit with a hypodermic needle, strangely enough, stained the nerves of the animal and no other part of the body. He asked himself, "If a proper narcotic can be mixed with methylene blue, will only the nervous system be affected and thus deaden the pain?"

This line of thought emphasized in Ehrlich's mind the question which is fundamental to modern chemotherapy. Is it possible to get certain chemicals that will have specific affinity to the bacteria and not the host cells they infect? With these questions, Ehrlich began his work on the discovery of Salvarsan, the first recognized chemotherapeutant.

Ehrlich and his Japanese assistant Dr. Ishiga experimented with the action of over five hundred different dyestuffs on more than two thousand mice inoculated with the parasite of African sleeping sickness, *Trypanasoma gambiense.* Finally, they found that the dyes, trypan-red and trypan-blue, were effective against trypanasomal infection in mice. From trypan-blue was evolved the substance 'Bayer 205' or germanin, which even today is the most powerful remedy against African sleeping sickness.

Ehrlich with a highly trained, experienced nineteenth century brain, and with the best equipment the country could offer him resumed the task begun centuries before by Paracelsus in a simple and primitive manner in treating syphilis.

The modern treatment of syphilis was evolved by the three great German scientists, Wassermann, Schaudinn, and Ehrlich. In 1905 Schaudinn discovered the causal organism: *Spirochaeta pallida* a light coloured spiral microorganism. In 1906 Wassermann discovered the test for ascertaining the presence of the disease and in 1910, after a decade of specialised work, Ehrlich found the chemotherapeutic remedy which serves as the basis for the cure of syphilis.

Ehrlich began experimenting with many chemicals which he hoped might destroy the *Spirochaeta pallida* without harming the body tissue. He pitched upon arsenic compounds for his investigation. With the help of a Japanese and a German assistant, Hata and Berthem, he tested patiently compound after compound using hundreds of experimental animals: mice and rabbits. He finished experimenting with 605 compounds and each of the compounds was given a name. Each of these was found to be deficient in one way or another; either they killed both the mice and spirochaeta or both were alive, or it was effective when injected but not when given orally and so on. For example, compound number 'one' was a sodium salt of acetic acid derivative of atoxyl. This compound, when injected on to a mouse suffering from syphilitic infections produced blindness of the animal. Certain other compounds tested produced symptoms of dropsy and jaundice.

However, in 1909, his 606th experiment, proved successful. The compound was called '606' for short. The real chemical of this great chemotherapeutic discovery was a compound of arsenic and benzene. It is a diamino-dihydroxy-arseno benzene-dihydrochloride; its chemical formula, $C_{12}H_{14}O_2 N_2Cl_2AS_2$, $2H_2O$.

On August 31, 1909, six rabbits, already in an advanced stage of syphilis when injected with compound '606', were cured. Paul Ehrlich created a new class of weapons against many deadly diseases, raising anew the hope of our ultimate victory over all germs. This has been proved true by the recent discoveries of antibiotics. With this compound called salvarsan the modern science of chemotherapy was born.

Ehrlich formally announced the discovery of salvarsan at the Medical Congress in Wiesbaden in Germany on April 19, 1910. But he was not prepared to lie back on his success. The discovery was not the ultimate for him. He rightly pointed out, "He who worships success can make the world neither good nor happy".

He did not pause to enjoy his success for he realised before salvarsan can be put to practical use there may be many practical problems. He found '606' oxidised very quickly and in this process the poison content of the compound increased and, therefore, salvarsan could not be stored too long. Thus, he was busy solving the minor problem before he could safely recommend salvarsan as a chemotherapeutant.

When Ehrlich died in 1915, von Behring, his professional rival, paid him a tribute thus: "None of us had done as much original work as you; you are the *magister mundi* in medical science."

4

Enter Sulphanilamide

WITH THE REVELATION of the chemotherapeutic character of coal-tar dyes many chemical industries in Germany organised elaborate programmes of research on these dye stuffs. The Bayers laboratories in Germany was a beehive of activity where scientists were working day and night on some dyes belonging to the 'azo' series (azo stands for nitrogen from the French word *azote*). Years and years of work yielded no fruitful results. In 1908, a doctorate student of the Vienna Institute of Technology synthesised an until-then-unknown coal-tar derivative called para-aminobenzene-sulphonamide, $C_6H_8N_2O_2S$. Using this as a base Bayer's laboratory produced a brick red dye and other azo sulphonamide dye stuffs. Nobody at that time was much interested in this compound. In the course of years other scientists began to consider seriously Paul Gelmo's contribution. Dr. Heidelberger and Dr. Jacobs of the Rockefeller Institute tested this compound among others in 1919, for destroying the pneumonia bacteria; para-aminobenzene-sulphonamide although effective *in vitro,* that is to say, when the microbe is grown in laboratory outside the human body, did not kill the bacteria *in vivo,* that is in natural living system, and hence no further step was taken.

Now we know that these two scientists had missed an important point. The very fact that sulphonamide does not kill but just inhibits bacterial growth was a point of significance in favour of sulphonamide therapy firmly established two decades later. The quintessence of sulphonamide therapy propounded subsequently lies in the capacity of the drug to cripple the bacteria and weaken it by interfering with its life process (bacteriostatic) and not kill it (bactericidal) and give enough time to the human body to allow the natural process of resistance to build up and throw out the bacteria. These facts were missed by Heidelberger and Jacobs.

In the year 1932, Bayer's laboratory took out a patent for a red dye, a derivative sulphonamide. This was called 'Prontosil' or an 'azo dye' containing sulphonamide group. This compound was overlooked for so many years as it had no effect on bacteria *in vitro.*

The astounding discovery of the century was made by Dr. G. Domagk, who, working in the dye stuff laboratory of Bayers, was investigating the therapeutic quality of various dye products including prontosil. He found that prontosil could cure streptococcal infection in mice completely. With patience Domagk and his assistants forced doses of prontosil down the throats of 1000 experimental mice. He repeated with rabbits and they too survived and recovered from streptococcal infection. He continued experimenting and he did not want to announce the chemotherapeutic potential of prontosil until he was quite sure. He was, however, forced by circumstances to use it on a human being—his own daughter. She had pricked her finger with a needle and blood poisoning had set in. She had caught a streptococcal infection. Physicians had given up hope of her survival when Domagk with rare courage gave her massive doses of prontosil, which had not yet been tried on human beings. She recovered completely. The medical world knew nothing of this astonishing event for three years, until in 1935 Domagk, the modest scientist, made a small scientific contribution in a journal and mentioned about the chemotherapeutic property of prontosil in strepto-

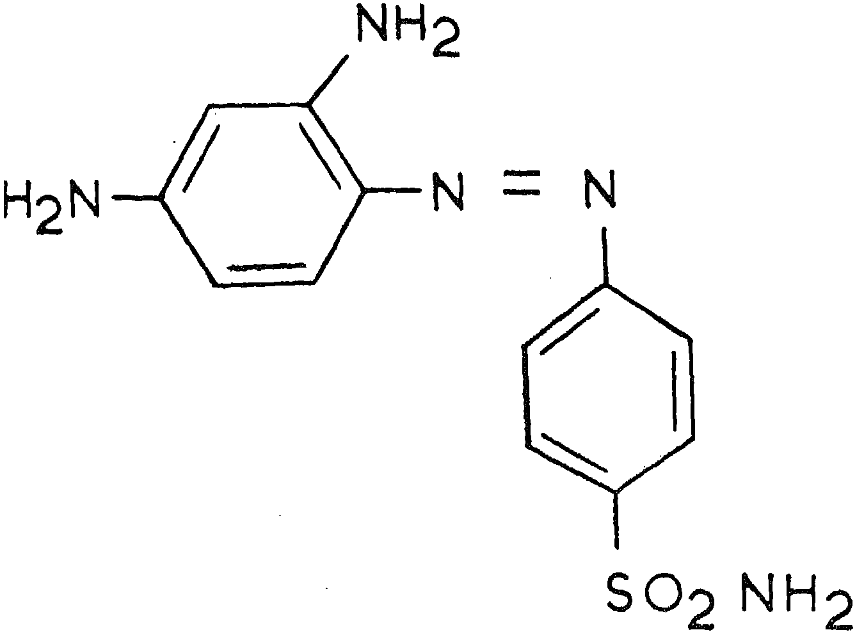

Fig. 1 Prontosil

coccal infection in mice. No mention was made of its effect on the human being. Work on prontosil spread throughout the world. It was soon found that it was a good remedy against all sorts of pus-producing cocci, cerebrospinal meningitis and gonorrhea.

Clinical and laboratory work showed that some synthetic derivatives of prontosils were more effective than prontosils. Experiments showed that sulphonamide (the substance that the Austrian scientist, Gelmo, synthesised), which was tagged on to the azo dye to make prontosil, was actually the fraction that was effective. It was soon shown that sulphonamide derivatives of prontosil were the most important part of the drug and prontosil was effective as a chemotherapeutic remedy only because, after its chemical breakdown in the body, sulphanilamide (as it is now called by the medical world), the effective portion of the original molecule was released; the substance which Gelmo synthesised was the real chemotherapeutant and not the azo dyes! The azo dye was a mere vehicle which released the sulphanilamide in the body! Sulphanilamide and its derivatives were being synthesised the world over and tried on many diseases. As sulphanilamide was not active against *Pneumococcus* bacteria causing pneumonia, May and Baker Laboratory in England tried a series of experiments and finally landed on a compound in their 693rd experiment which was successful. This is a combination of 'sulphanilamide and pyridine'. The fact that 692 experiments preceded the final discovery reflected the extent to which, in 1938, the whole science of chemotherapy was still in trial and error stage of development.

In 1940, a further step was reached in the science of chemotherapy when it was shown how exactly sulphanilamide prevents bacterial growth. This gave a reasonable understanding of the mode of action and thereby a rational approach of evolving more chemotherapeutants became a possibility. Before we proceed further, let us try to understand the bacteria, their range of shape, size, structure, nutrition and growth.

Paul Ehrlich

G. Domagk

E. B. Chain

Howard Florey

Plate 2.

5

A Close Look at the Enemy

By the time the basis of sulphanilamide inhibition of bacterial growth was established in 1940 the science of bacteriology had advanced far beyond from the mere sighting of the bacteria by Leeuwenhoek in 1677. A German professor of Botany, F. J. Cohn, completed a magnificent book in the later half of the nineteenth century which marks the real beginning of the study of bacteria. Cohn's observations, using the compound microscope, were so accurate that we can still make use of his illustrations and descriptions of bacteria, although much progress has been made since then on studies of fine structure and reproduction.

Micro-organisms occur everywhere; in soil, water and air around us. There are different types of micro-organisms. Most of these are harmless, some are helpful and are used in industry, while a few are harmful. It would be wrong to believe that all the infectious diseases of man and animal are caused only by bacteria and hence chemotherapy is directed only against bacteria. Some of the important groups of microbes that cause disease are protozoans, moulds (fungi), ray fungi (actinomycetes) bacteria and viruses.

Protozoa are unicellular organisms, but unlike bacteria, their protoplasm is differentiated into cytoplasm and nucleus; malaria and amoebic dysentry are caused by protozoan parasites.

The fungi are tubular branched, filamentous organisms, barring the yeasts and yeast like unicellular forms—without chlorophyll, and some of them cause diseases of plants, animals and man. The common ringworm, as well as certain mycotic infections of the lung simulating tuberculosis, are a few examples of fungal diseases of human beings.

The actinomycetes are also made up of branched filaments like fungi but these are very thin and they have many physiological characters, similar to bacteria. The filaments break up into fragments and they resemble rod shaped bacteria; bacillus. The micro-organisms causing tuberculosis and leprosy belong to this group, look like bacilli and hence are referred commonly as bacilli. Many species of Actinomyces cause infections of man and go under the name of actinomycoses. But species of the genus *Streptomyces* has been most beneficial to mankind; they produce some of the most potent antibiotics that are available today.

Bacteria are unicellular forms without chlorophyll and a discreet nuclei. They are divided into, cocci, bacilli, vibrios and spirochaets. Cocci are spherical bodies and common examples which affect man, include staphylococci, streptococci, pneumococci, gonococci, meningococci. Staphylococci are found in boils, carbuncles, abscesses and occasionally in a form of pneumonia. Streptococci, cause such diseases as tonsilities, erysipelas, cellulitis and child birth or puerperal fever. Pneumococci cause one form of pneumonia. Gonococci cause gonorrhoea and meningococci give rise to one variety of meningitis. Bacilli are straight rod shaped organisms, some of them move about with one or more whip like structure called flagellum (flagella—plural); common examples of bacilli pathogenic to man include those responsible for diphtheria, typhoid fever, dysentry, anthrax, tetanus and gas-gangrene. Vibrios consists of small actively motile organisms in the shape of a comma, of which a good example is the one that causes cholera. Spirochaetes are thin spiral quickly moving filaments; examples include the variety responsible for syphilis and the one which causes relapsing fever.

Finally, there is a group of organisms called viruses which are so small that they cannot be seen under the magnification of the ordinary microscope. Some of the virus diseases which affects man are the common cold, influenza, measles, mumps, chickenpox, small pox and poliomyelitis.

It was because bacteria form only a part of microscopic life that Sedillot, in 1878, introduced the term 'microbe' to describe any living organism so small as only to be visible under the microscope and thus the science of bacteriology forms but a part of the science of microbiology.

Until the structure and reproduction of viruses were classified, bacteria were considered the smallest living things. Bacteria are measured in microns (one thousandth of a millimeter). The bacteria range in size from 0.2 micron (μ) to

2.0 micron (μ) in width and from 2.0μ – 10.0μ length. This term length and width does not apply to cocci which are spherical.

Bacteria appear at the level of cellular organization and immediately above the viruses. In many ways, however, a bacterium is much simple in structure than the cells of multicellular plants and animals. The bacteria do not have nuclei of the type found in cells of higher plants and animals. In the cells of the more complex organism, the nucleus contain a nucleolus or two, is bounded by a visible membrane and divides by a special process called mitosis. In bacteria, there are no nucleoli, no nuclear membrane and division is by a simple method. The nuclear material or Deoxyribose nucleic acid (DNA) similar to that in the chromosomes of higher plants and animals, is in the form of long linear strand, packed tightly. The DNA molecule is about 3 millimicron (mμ) wide and 1000 micron (μ) or 1 mm long. A bacterial DNA molecule is about 500 times as long as the bacterial cells that contains it. Other structures of bacterial cell are vacuoles, ribosomes and granules of stored food. The bacteria are classified as Gram positive or Gram negative, on the basis of certain differential staining. After staining by this method the organisms are found to be either violet or red. All the stainable spiral organism, about one third of cocci and one half of the bacilli appear red. This empirical discovery of the Danish Bacteriologist, Gram, appears even today as one of the simplest and most useful techniques in all microbiology.

A thin film of the bacterium is placed on a glass slide and fixed by heat. It is then treated with gentian or crystal violet for a minute, rinsed quickly in water and covered with Gram's iodine solution for one minute. Following another quick rinse in water the slide is decolorised with alcohol or a mixture of acetone and ether, washed thoroughly and counterstained with safranin for 10 seconds, washed with water and dried. The organisms which retain the violet dye are called as Gram-positive and those which are decolorised and counterstained with the red dye (safranin) are called Gram-negative.

The differential staining appears to be due to the differences in the nature of cell wall. Studies have shown that the cell wall as well as protoplasm of both Gram-positive and Gram-negative stain with crystal violet. Actually some of the Gram-negative bacteria can bind more crystal violet than Gram-positive. The organism retains the same size after treatment with iodine. With brief decolorisation with alcohol the blue dye is removed from the Gram-negative organisms but not from Gram-positive ones. On counterstaining with safranin the decolorised Gram-negative stains red. The treatment with iodine produces a crystal violet—iodine complex in both Gram-negative and Gram-positive organisms. The process of alcohol dehydration after iodine treatment appears to dissolve away much of the lipid from the cell wall of Gram-negative bacteria, after which the crystal violet—iodine—complex escapes through the enlarged cell wall pores. In contrast, the dehydration by alcohol reduces the size and pores in the cell wall of Gram-positive bacteria and makes decolourization of the cells much more difficult. The Gram-reaction is indicative of a more profound difference between the two groups, than a mere physico-chemical reaction to dyes.

The cell wall under normal conditions, encloses and is in direct contact with cytoplasmic membrane. The wall represents 20 per cent of the dry weight of the organism and varies in thickness from 10 to 25 $m\mu$. It contains pores approximately 1 $m\mu$ in diameter which admits small molecules to the cytoplasmic membrane.

The wall of Gram-negative bacteria are chemically more complex than those of Gram-positive species. The walls of Gram-positive bacteria contain a small variety of amino-acid (the building blocks of proteins in all living system) amino sugars, and polymerised sugar components as mucopeptides, polysaccharides and the teichoic acids. Splitting up of these by hydrolysis reveals several substances not generally encountered in cells of higher organisms.

In the Gram-negative bacteria also, a rigid muco-peptide layer chemically similar to that in Gram-positive is seen but it accounts for only 10 to 20 per cent by weight. In addition this is reinforced by lipoproteins and lipo-polysaccharides.

The presence of structural compounds in the cell wall unique in that they are not found in the tissues of higher forms of life, makes the bacterial cell wall an excellent target of attack by chemotherapeutant, without harming the higher organism that harbours the bacteria.

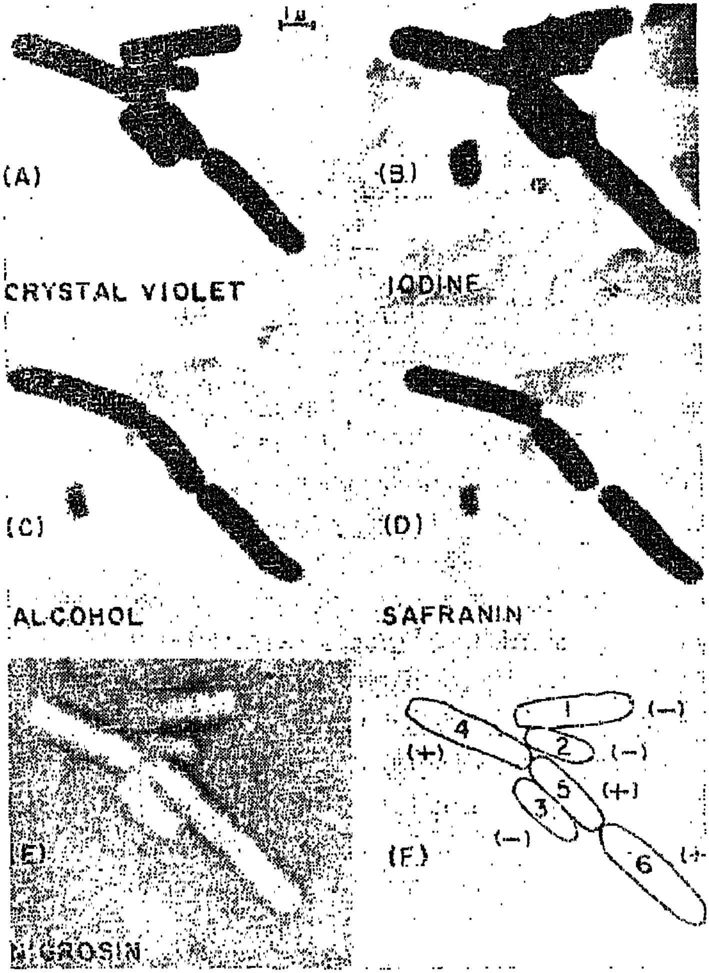

Plate 3. Gram stain of *Bacillus subtilis*. The appearance of a group of cells following each step of the gram stain procedure (A – D) and nigrosin-negative staining (E). The group contained both gram-positive (dark-appearing) and gram-negative (light-appearing) cells.

6

The Enemy Grows and Multiplies

LIKE ALL OTHER LIVING THINGS, bacteria need energy for their growth, maintenance and multiplication.

Most bacteria are heterotrophic in their nutrition. They cannot synthesize their requirements of organic compounds from simple inorganic substances. They require a multitude of complex organic materials such as amino acids, glucose and vitamins in addition to inorganic substances.

But some kinds of bacteria are autotrophic. They can synthesize their needs of organic compounds from simple inorganic substances. A few of them are photosynthetic and have pigment similar in molecular structure to chlorophyll of green plants.

In multicellular organisms, the cells produced by cell division generally remain together and larger organisms are the result. A bacterium is, however, a single celled organism. When it divides and produces two cells we have two organisms where we started with one. This kind of reproduction is a sexual, meaning that it does not involve union of sex cells in the manner characteristic of so many animals and plants. When a bacterium divides the division of the cell is preceded by the division of the nuclear material and the type of division is called fission. A bacterium can divide and the two daughter cells can grow and start dividing again in 20 minutes to form 4 cells; each of the four then divide to form 8, then 16, 32, 64, 128, 256 and so on, doubling every twenty minutes. If this kind of increase, called geometric increase, were to continue, once every twenty minutes, for 24 hours, a mass of bacteria weighing approximately 2,000 tons would be produced from a single cell. Actually this would never happen, because the bacteria would soon run out of sufficient water and nutrients needed for their reproduction. As a bacterial population grows, the bacteria usually produce substances such as alcohol or acids, which are poisonous to them. The accumulation of these can cause a decrease in the rate of reproduction so that the number of new cells produced almost equals the number of cells that die.

Exchange of nuclear material between two bacteria has been demonstrated recently confirming the existence of a simple type of sexual reproduction in bacteria.

Bacteria prefer nutrient soup

Micro-organisms are so small that it is impossible to handle them individually. We normally handle in laboratory a cluster of them which is also referred to as a colony. A colony is a group of micro-organism of the same type developed from a single organism.

In nature, many kinds of micro-organisms are found growing together, but when we grow or culture micro-organisms in the laboratory, we usually want to separate the different kinds and grow each kind or species as a pure culture. If we have a pure culture of each pathogenic bacteria we can test new drugs against each one of them and find out its action.

In the early days of microbiology, nutrient broth or soup containing all ingredients necessary for growth was used. If Pasteur was interested in the micro-organisms involved in wine production, he would put a tiny drop of wine in one of these culture solutions. Because the original drop would frequently contain more than one species of micro-organisms it would be difficult to obtain a pure culture. The problem of separating one species of bacterium, from the others, so that it can be grown in pure culture, was solved in the laboratory of Robert Koch. He discovered that a solid rather than a liquid culture medium could be used to isolate pure cultures of micro-organisms. Koch mixed his nutrient soup with gelatine to make it solid. The sterilized mixture of nutrient medium was poured into Petri dishes. (The Petri-dish named after Koch's student Petri comes in two parts. Each part is a flat bottomed round glass dish with a small rim.) The culture medium is poured into the smaller dish and it is covered by the bigger dish—somewhat like a soap box—which prevents micro-organism entering in from the air. This bacterial suspension can be mixed with the nutrient medium and poured and the individual cells would stick at different locations on the surface. The invisible cells start multiplying in geometric progression and become visible in particular locations, as a colony. Alternatively using a needle, a small drop of bacterial suspension can be streaked on the surface of the solid medium, which gives out individual colonies.

A woman by name Mrs. Hesse suggested to Robert Koch *agar* an extract of sea weed as a substitute for gelatin. While gelatin is liquified by certain bacteria agar is not attacked by bacteria. Again gelatin melts at a rather low temperature—even at room temperature in the tropics.

Agar does not melt until a temperature of 90°C is reached and the liquified agar does not solidify until it is cooled to 40°C. (This property is called Hysterisis—water solidified to ice at 0°C and ice melts to water at 0°C. Compare this with agar!) The fact that agar can be melted at 90°C and also once melted does not solidify until cooled to 40°C has made it possible to be used in refining methods of pure culture study. In all culture studies very strict precautions must be taken to prevent contamination, by sterilizing glass ware, using antiseptics for personal cleanliness and disinfectants at the places of work.

Using such techniques scientists have now obtained pure cultures of almost all pathogenic bacteria and this is a very essential pre-requisite in the studies leading to the discovery new chemotherapeutants and antibiotics.

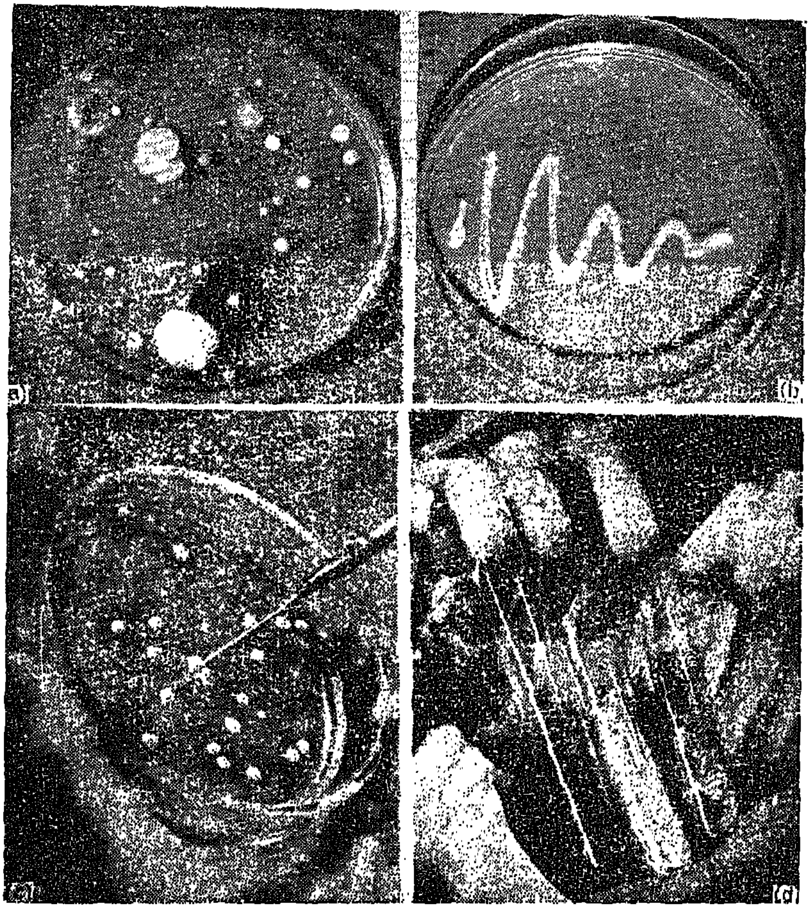

Plate 4. (a) Colonies of bacteria developing on agar medium in Petri dish — soil dilution method
(b) Colonies of bacterium developing on agar — streak method
(c) Isolating a pure culture of an actinomycete from one of the colonies developed in Petri dish
(d) Maintaining pure cultures of microorganism in test tube slants.

7

How Sulphanilamide Knocks out Bacteria

ALTHOUGH MANY YEARS have elapsed and much progress has been made since Ehrlich first introduced the term "chemotherapy", one is still impressed with the fundamental soundness of his general concepts. As used by Ehrlich the term "chemotherapy" denotes the treatment of parasitic disease by a direct chemical attack upon the invading organism. Chemotherapeutants do not act primarily on the host but combine chiefly with certain systems of the parasitic organisms, thus focussing attention on the parasite rather than the host that harbours it. This was well presented by Ehrlich in his chemoreceptor or side chain hypothesis.

Many organic and inorganic substances had been known for years to inhibit the growth of micro-organism, but their indiscriminate affinity for the host proteins had limited their use in most instances to topical application (antiseptics) or sterilization of inanimate objects (disinfectants). Advance in the field of chemotherapy was slow and with the advent of sulphanilamide, the idea of selective action and thereby host tolerance to chemotherapeutants became a reality.

It may be profitable at this point to illustrate some of the general principles involved in the mechanism of action of one of the most successful group of chemotherapeutic agents—the sulphanilamides.

Bacteria are organisms potentially able to synthesize their structure and accesary components from simple compounds, though they may have lost the power to carry out one or more stages of essential synthesis. In such cases the product of the particular stage of synthesis, which fails, becomes a growth factor which must be added to the artificial nutrient medium. These growth factors, however, are members of a much wider class of essential metabolites without which the micro-organism would not grow.

Shortly after the discovery that sulphanilamide (p-aminobenzene-sulphanamide) would inhibit the growth of many kinds of bacteria, it was recognized that the harmful effects of this agent could be nullified by a component of the nutrient soup in which the organisms were grown. Materials as extracts from yeast or even streptoccoci when added would retard the action of sulphanilamide. Woods attempted to isolate this substance from yeast extract and he was able to show that it was an amphoteric compound with high activity when compared to the amount of sulphanilamide which it antagonised. Very small amounts of the partially purified extract nullified the bacteriostatic action of moderately large concentration of the drug. Because it was known among enzymologists that certain enzymatic reactions could be inhibited by addition to the system of a compound related in structure to the normal substrate, Woods suggested that the antagonist to sulphanilamide, which was found in living cells, was a structural analogue of sulphanilamide. In fact, it was shown to be p-aminobenzoic acid (pABA). At that time p-aminobenzoic acid was not known to be a metabolite, but shortly after his postulation of the role of this compound in metabolism abundant proof from various sources substantiated his claim.

p-aminobenzoic acid (pABA) was very active in overcoming the inhibition of growth of bacteria which sulphanilamide caused. Further more the antagonism was competitive. Thus, if 50 microgram of sulphanilamide was needed to stop the growth of a strain of streptoccocci when 1 microgram of pABA was present, 10 times as much was needed when 10 microgram of pABA was present. Thus, the toxicity of sulphanilamide to the bacteria could not be defined in terms of concentration of it in the nutrient solution but rather had to be stated, in relation to the amount of pABA present. This competitive character of the antagonism was an important feature which has now assumed prominence in the interpretation of this phenomenon.

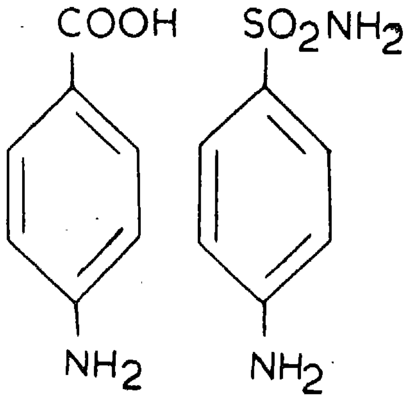

Fig. 2 (a) Para-aminobenzoic acid (b) Sulphanilamide p-aminobenzoic acid is an essential ingredient of another essential metabolite folic acid which is also called Pteroylglutamic acid (PGA). When sulphanilamide is present in the medium the bacterial enzyme in mistaken identity takes in sulphanilamide in the place of pABA as the substrate. While pABA would have combined with the enzyme to yield folic acid (PGA), sulphanilamide once combined to the enzyme forms a complex and blocks any further process and no PGA is formed. As PGA is essential for bacterial growth the bacterium is virtually starved of this essential substance and growth is inhibited. One can overcome the deficiency created by sulphanilamide, by adding sufficient concentration of the substrate pABA or the end product of the enzyme reaction folic acid itself.

This enzyme block by a structural analogue of a metabolite can be best explained by a lock and key mechanism. If a lock were to have two keys, one its original and another very similar to the original, then with the former one can open the lock, but with the latter one gets stuck onto the lock and is unable to open the lock or get the key out of the lock. A similar situation exists between metabolites and antimetabolites or in this instance between pABA and sulphanilamide.

If folic acid is needed for animals and man, one may ask whether sulphanilamide which inhibits bacterial growth by preventing folic acid synthesis and thereby creating a deficiency of this vitamin, will not produce the same folic acid deficiency in man, when he is treated with sulphanilamide for bacterial infection. In the case of bacteria, in which sulphanilamide causes inhibition, it has been shown that folic acid is synthesised by the bacterium concerned and in the presence of the drug, folic acid synthesis stops. In the case of man and animals they cannot synthesize folic acid and they have to be supplied any how with an external source of folic acid. In other words, man and animal show a deficiency for folic acid similar to what the bacteria show, in the presence of sulphanilamide. Hence, sulphanilamide can produce no folic acid deficiency in man and animals. This makes the action of sulphanilamide selective. By the same token, it could be

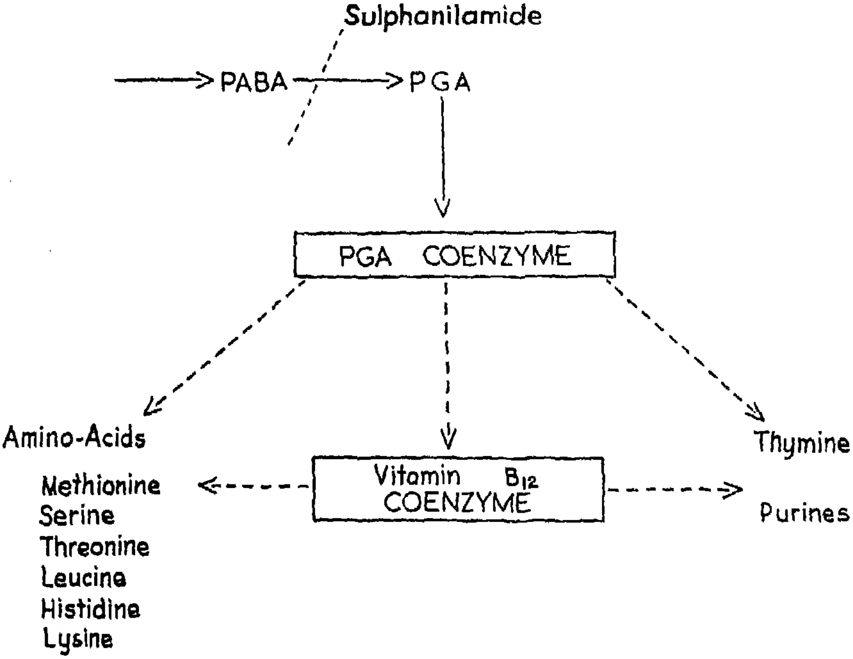

Fig. 3 Sulphanilamide inhibition of para-aminobenzoic acid

shown that those bacteria that cannot synthesize folic acid but has to be supplied with an external source of folic acid, are also not sensitive to sulphanilamide.

This selective action, is one of the prime requisites of a useful therapeutic agent. Although this conviction was prominent in the early experiments of Ehrlich with arsenical compounds, sufficient knowledge was not on hand at that time, beyond the finding that susceptible organism somehow bound the drugs. The hypothesis arising from the discovery of p-aminobenzoic acid fell short of yielding useful drugs immediately, partly because of the lack of understanding of this problem of selective action; practically the only success among the many early failures in the use of antimetabolites as chemotherapeutant in infectious diseases, was, that of pantothenic acid analogues in malaria. This was based on the chance observation that the analogues were harmless to animals but still toxic to micro-organism. Thus, phenylpantothenate and dibromosulfanilide, the two analogues of pantothenic acid, proved to be good drugs in the cure of malaria. They produced no deficiency of pantothenic acid in man and experimental animals.

Another drug that was discovered in 1952 with the antimetabolite approach, is isonicotinic acid hydrazide, called isoniazid for short, used in therapy of tuberculosis. Isoniazid is effective in very low concentration and is very selective in its action against *Mycobaterium tuberculosis,* the causal agent of this disease. The drug is bactericidal and is active only on the growing organism. The combination of isoniazid with the antibiotic streptomycin (this will be referred to later) is the mainstay in the therapy of tuberculosis. In postulating the mode of action of isoniazid many views have been put forward with suggestive evidence. Structural relationship between isoniazid and the vitamins of the 'B' group, niacin (nicotinic acid) and pyridoxin suggested that it might act as an antimetabolite of the two vitamins. Isoniazid produces in man Vitamin B 6 (pyridoxine) deficiency when given over extended period of time and this can be overcome by external administration of this vitamin to the patient. Here is an example of a drug whose action is not selective but yet could be used because of its higher toxicity to the micro-organism than to man.

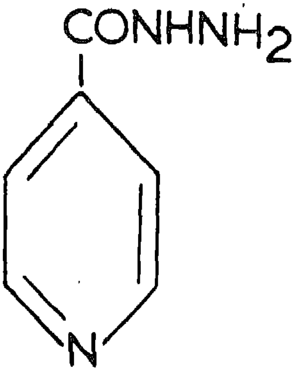

Fig. 4 Isonicotinic acid hydrazide

We have come a long way from Ehrlich's first enunciation of the concept of chemotherapy. Yet the number of chemotherapeutants that can claim success in combating diseases are very few. The basic principle of designing antimetabolites to attack the micro-organisms selectively is now being applied against viruses, but not with much success. We shall refer to this later on. Designing of more powerful drugs against bacteria on the basis of the antimetabolite concept was an arduous task; and before any worthwhile compound was discovered, the microbes themselves offered to man, a powerful therapeutant—pencillin.

8

The Miracle Drug from A Mould

On the 20th May 1940, during the second world war, when invasion of England appeared imminent, Dr. Heatley, an assistant to Dr. Florey, then Head of the Sir William Dunn School of Pathology, Oxford, infected three groups of mice with different organisms; half of them were also given a substance that had been extracted from a mould and purified with great care by the Biochemist Dr. Chain. Dr. Heatley sat up all night watching with excitement the treated mice survive and the untreated die. Next morning, Florey and Chain were jubilant when they saw the result. They realised that they had in their hands an extremely valuable agent to destroy bacteria.

On July 1, they injected a large number of streptococci into fifty mice. Half of them were given this substance extracted from the mould, the other half were left untreated as controls. Florey, and his assistant were awakened by an alarm clock every two hours of the night to observe the animals.

All the 25 controls were dead in sixteen hours. Twenty-four out of twenty-five of the mice treated with this new substance from the mould, had survived. It seemed almost a miracle and the result was factually reported in the medical journal, *The Lancet* on 24th August 1940. This substance extracted from the wound was nothing else than penicillin which arrived on the scene in 1940, 12 years after its initial discovery, holding high promise as a therapeutant.

Twelve years earlier, in 1928, Alexander Fleming working in the bacteriological department of St. Mary's Hospital, England, noticed one day that his staphylococcus culture in the Petri-dishes had become contaminated, with a greenish blue mould, later identified as *Penicillium notatum,* not very different from that which collects on bread, or damp shoes. Moulds or fungi, begin to develop when a tiny invisible spore which is floating around us in the air alights on any food base. On the day Fleming discovered this mould, he noticed, however, that a strange thing had happened. The mould was growing at the edge of the Petri-dish and around the mould the culture medium was free of staphylococci. The bacterium had been destroyed by the mould. Fleming argued that the mould was producing a chemical substance which killed that particular bacterium. Professor Fleming had described his early work on the bacteriolytic property of this substance from the mould *Penicillium notatum* and he clearly visualised the possibilities of penicillin and suggested that it might prove to be an effective agent for counteracting bacterial infections.

Fleming knew that the nutrient solution in which *Penicillium notatum* was grown was more potent than carbolic acid, the great antiseptic of those days. He also found that penicillin was non-toxic to animals. Fleming also noted that penicillin could not be easily extracted as it was chemically unstable. Incidentally, it is of historical interest to know that Fleming had published his first papers on his early experiment with Penicillin in 1929, quite six years before prontosil, the father of sulphanilamide became known! Yet nothing substantial could be done until Florey and his collaborators took up the challenge in 1939.

In 1930 at the London School of Hygiene and Tropical Medicine, Professor Raistrick began experiments in concentrating penicillin from solution. He grew the mould in a synthetic nutrient solution and noted that penicillin, as Fleming observed, was unstable. When the ether extract was evaporated in air, the potency of penicillin was lost. Thus, up to 1933, when Raistrick stopped his experiment, all findings were discouraging for it appeared that chemical methods to concentrate penicillin led to its destruction.

At St. Mary's Hospital, rested for nearly 10 years, penicillin in the crudest form; however, the culture of the mould was sent to different laboratories on request. For over a decade from 1929 to 1940 all the penicillin produced in the world came, from descendants of that one spore that blew in through the window of St. Mary's Hospital in London and sneaked into the Petri-dish of Fleming.

It was at this stage that Oxford School of workers entered the scene. In 1938, the Oxford School of Workers, lead by Florey started working on antibacterial substances. They chose lysozyme, a substance present in tears, as well as other secretions of the body. Chain, the biochemist of the Oxford School who was entrusted with this task, went through the literature on this subject and noticed Fleming's contribution on lysozyme. He also noticed the report of Fleming on penicillin, *i.e.,* it could kill dangerous bacteria without harming the body. He also read the attempts that had been made to purify this drug without success. Chain wanted to take up this challenging problem but, money was needed. Florey and Chain submitted a scheme and got 5,000 dollars from the Rockefeller Foundation of America. It was thus that Chain started his work on Penicillin in 1939. Starting with the mould which obviously had been sent by Fleming to the Oxford School a few years before, Chain after hard work and surmounting various difficulties in culturing the mould, got the fairly pure sample which as mentioned earlier at the beginning of this chapter had cured the 25 mice of staphyloccal infection. Penicillin came of age on August 24, 1940, when this was reported in the medical journal *Lancet*. When Fleming read the report he was both delighted and surprised, as he had no idea that this work was going on at Oxford. Now when he had almost given up hope of getting penicillin purified by chemists, it had been done for him without asking. The Oxford Scientists had been encouraged to do this, as Fleming's account of penicillin had been precise in his published reports. The Oxford School had not got in touch with him at any stage and in fact, when Fleming went to Oxford to see the workers at Oxford, Chain had the shock of his life because he had assumed Fleming was dead.

The first ever injection of penicillin on a human being was done on February 12, 1941. A policeman of Oxford got staphylococcal infection of his blood and was seriously ill with blood poisoning. Sulphanilamide could not cure him as the Staptylococci were not sensitive to it. A small amount of penicillin was injected and he recovered sufficiently and doctors held high hopes of his recovery. Although the bacteria had been reduced in number it was not completely eliminated. Some more penicillin was needed and the doctors ran short of supply of this drug. The man's condition again deteriorated and before the chemists could prepare some more of the sample he died on March 15, 1941. This was a tragedy because it was obvious that with sufficient penicillin, the man's life could have been saved. After some more supplies were got ready three more serious cases were treated with success and it was clear to everyone that here was a remarkably powerful weapon against man's enemy, the bacteria.

Florey realised that the only way to produce this substance in large scale and save the ailing humanity was to produce it on a commercial scale. In England all most all industrial chemists were committed to war efforts and hence Florey decided to try his luck with the American chemical industry. This lead Florey and Heatley in June 1941 to journey to the USA risking the German submarines in the Atlantic. War was at its severest and the Oxford workers did not want the secret of this mould to go the German enemy, if they were caught. It is said, they dipped their coats in a culture of *Penicillium* and carried the culture thus, instead of in a regular flask or a tube, to escape confiscation if and when captured by the enemy.

In New York, Florey met Thom who had eleven years before helped Fleming to identify the mould as *Penicillium notatum.* He was now head of a section which was studying fungi at the Northern Regional Research Laboratory at Peoria, Illinois. Thom introduced Florey to Dr. Coghill who was the chief of the Division of Fermentation. There was a free and frank exchange of views between the American and British Scientists. At no time did any of the British workers seek to protect their discovery by taking out a patent as everyone agreed that a substance of such vital use to mankind ought not be subject of personal profit.

The Americans decided to change the nutrient solution in which *Penicillium* could be grown, to see if higher yield could be obtained. Corn (maize) steep liquor which was available in plenty proved to be of great value in enhancing penicillin production. In addition a woman was employed to go out to the market in Peoria everyday and bring back mouldy fruits and vegetable in the search for other types of *Penicillium* which may produce more penicillin than the original one. This lady later nicknamed 'Mouldy Mary' came one day with a mouldy cantaloup melon and the mould which was identified as *Penicillium chrysogenum* gave a remarkably high yield of penicillin. It is of interest, that it is from the mould from the decayed melon of the Peoria market place in Illinois, USA, that most of our penicillin was derived until synthetic penicillin came into the market a few years ago.

Florey travelled in America and Canada. As America was not then at war, he could get two firms interested in large scale production of penicillin and send it to England for research purposes.

During the time Florey and Heatley were in America, Chain continued to direct the work at Oxford and his team perfected a method of extraction which enabled them to supply small doses of the drug to cure some of the seriously wounded soldiers in the battle field. The miraculous cure of these soldiers for whom death was a certainity made doctors sing praise of the Oxford School of Workers.

In America, the progress was slow to start, but all the workers were most enthusiastic and before long, the technical difficulties were overcome and large quantities of penicillin were released. By 1944, penicillin could be released for treatment of people throughout the world.

The world payed homage to the men who made this possible Fleming, Florey, Chain, Heatley and other Oxford School of Workers. Never before in history was so much owed by so many to so few.

A quick and simple method of evaluating the concentration of penicillin in crude samples was evolved early in penicillin assay by Heatley and he introduced a standard measurement of Penicillin, so that research workers everywhere knew the strength of the solutions they had and its effect. This measurement is called Oxford Unit and for short 'Unit'. This unit is the amount of penicillin which when dissolved in 50 ml of meat extract under standardized conditions, just inhibits the growth of staphylococcus. If, for examples some other germ needs a solution five times as powerful to be inhibited, then we say it takes five units to inhibit this organism. One comes across often the term 'unit' being used on samples of

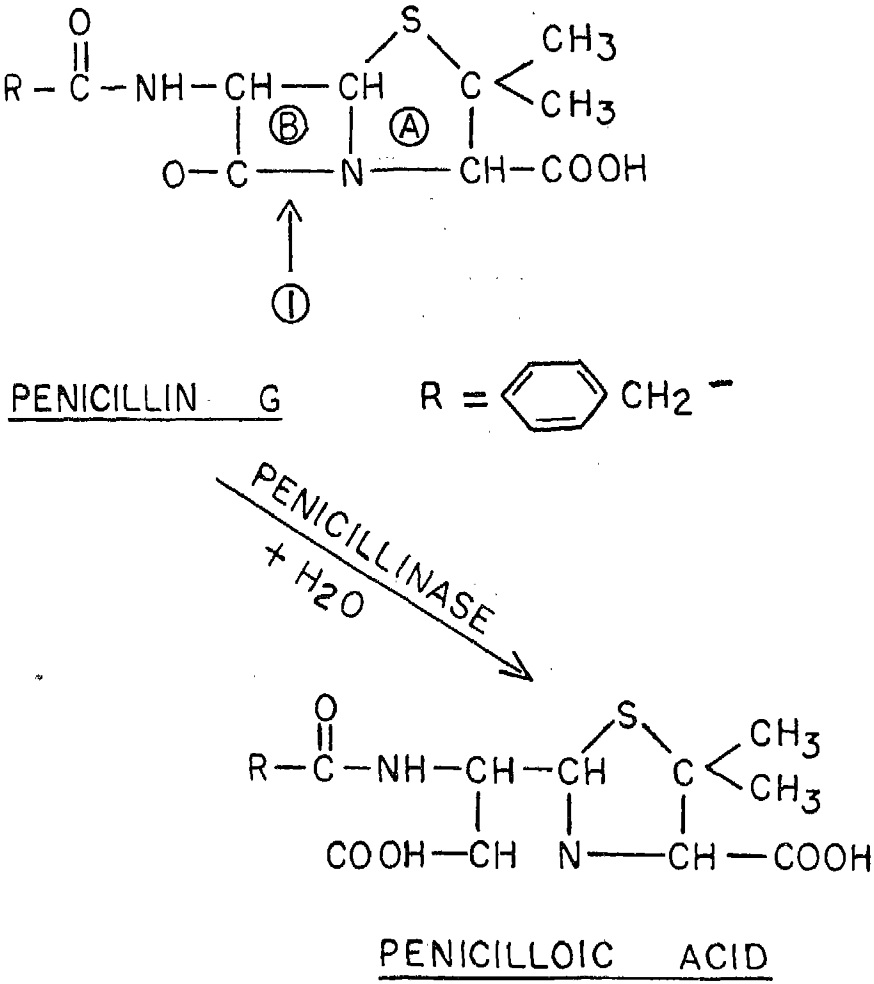

Fig. 5 Structure of penicillin G.

A = Thiazolidine ring

B = Beta-Lactam ring

I = Site of action of enzyme penicillase penicillin we purchase and this refers only to the strength of the drug with reference to this standard set up by the Oxford School of Workers.

The chemistry of penicillin was worked out during World War II as a result of the joint efforts of many laboratories in the USA and England. The basic structure of penicillin consists of a nucleus of a thiazolidine ring fused to a beta-lactum ring to which, in turn is attached a side chain that determines some of the specific properties of penicillin.

The introduction of penicillin revolutionized the treatment of many infectious diseases. It is effective against practically all of the gram-positive organisms as well as gram-negative cocci and spirochaetes. Remarkable success has been achieved with penicillin in streptococcal and staphylococcal infections, in subacute bacterial endocarditis, gonorrhea, pneumonia, syphilis, gas gangrene, actinomycosis and a number of surgical conditions.

One of the hazards of the use of antibiotics is the development of a strain of the organism that can tolerate the antibiotic drug-resistant strain. In the case of penicillin this has been limited, luckily, to staphylococci. Although staphylococcal infections responded to penicillin therapy initially, by the year 1955, as many as 90 per cent of the staphylococcal infections in hospital conditions were found to be associated with penicillin-resistant strains.

These staphylococci are now known to produce an enzyme, penicillinase, which splits the beta-lactum ring of the penicillin nucleus, thus rendering the drug antibiotically inactive.

In 1957, the 6-aminopenicillianic acid was successfully synthesised and as a result of this, new groups of semisynthetic penicillins have been introduced in the market, giving hope of treating infections with resistant strains of staphylococci. Among the penicillins resistant to the enzyme penicillinase are methicillin, oxacillin, cloxacillin, diphenicillin and nafcillin.

Semisynthetic penicillin, like ampicillin, offers promise in the treatment of certain infections of *Salmonella typhosa*, *S. paratyphi* A, *S. paratyphi* B and *S. typhimurium*, typhoid variety of fevers caused by gram-negative rod shaped bacteria.

Mode of Action

All the penicillin varieties appear to have similar action, inhibiting bacterial growth. At low concentration, penicillin is bacteriostatic, and bactericidal at higher concentrations. The inhibition occurs only under conditions of active growth of the organism concerned. The shape and size of bacteria are affected, resulting in bizzare morphology under the influence of penicillin. This gave the clue to the site of action of penicillin. It is now reasonably certain that penicillin inhibits one or more enzymes involved in the synthesis of the cell wall. The most suggested evidence of the enzymatic reaction site of penicillin is that it inhibits a process involved in the insertion of N-acetylmuramic acid into the mucopeptide polymer, one of the ingredients of the cell wall of all bacteria. Once the cell wall synthesis is impaired the new cells that are formed are sensitive to osmotically variable gradients of the living system and are unable to live in such an environment.

Allergy to Penicillin

Vary high levels of penicillin can be tolerated by man. There are, however, a number of cases of allergic or hypersensitive reaction leading to death. According to recent concepts this sensitivity is caused by certain derivatives of penicillin associated with the pure molecule of penicillin. A skin test done with the penicillin to be injected to see hypersensitive reaction, if any, is a useful safeguard against such unfortunate fatalities.

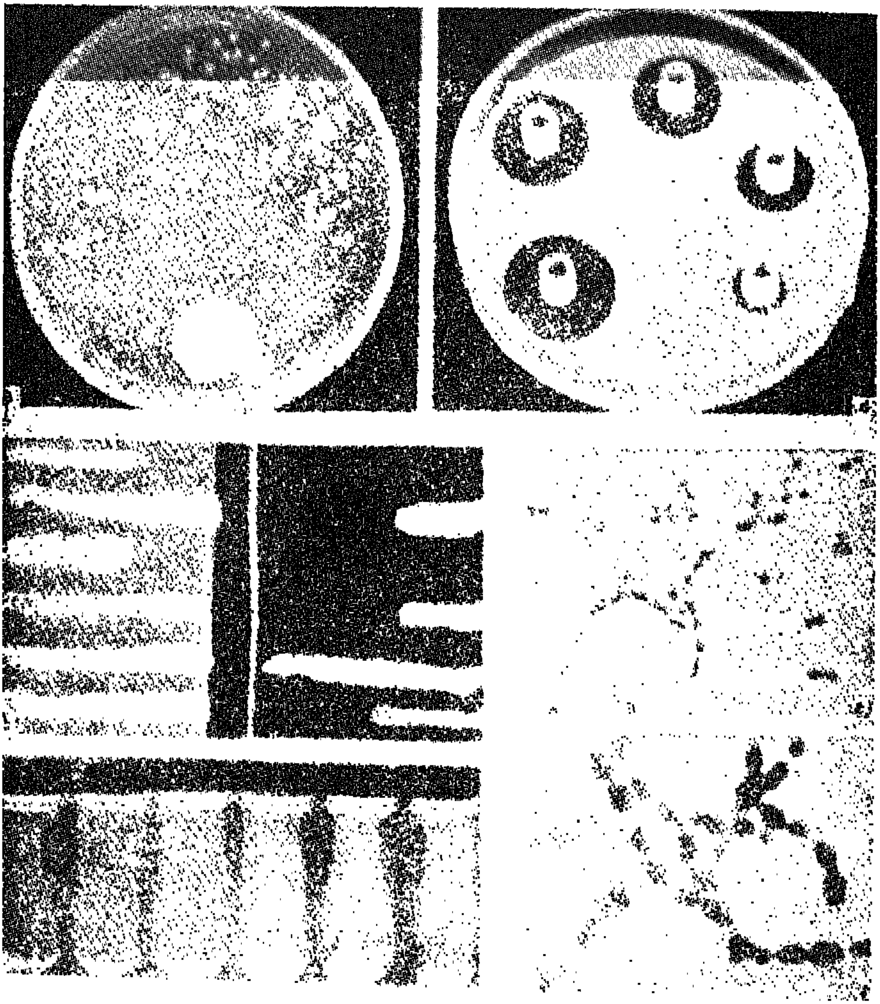

Plate 5. (a) The original plate of Sir Alexander Fleming. Note the growth of the contaminant mould *Penicillium* around which the colonies of staphylococci are slowly disappearing indicating the bacteria-killing property of the mould.

(b) A method of screening sensitivity of different bacteria to an antibiotic. In the central 'gutter' cut in the agar is a crude solution of penicillin which diffuses in the agar. On either side are streaked different species of bacterial cultures; gram positive on the right and gram negative on the left. Those which are sensitive are prevented from growing up to the gutter and those that are not sensitive grow up to the gutter.

(c) Serial dilutions of penicillin in nutrient soup containing staphylococci (from left to right, 1/16, 1/64, 1/256, 1/1024, 1/4096 and control); growth of the bacteria in the tubes on left has been suppressed by penicillin.

(d) Heatley's assay method or the agar plate method for penicillin assay. Porcelain cylinders are placed on the surface of agar medium sown with staphylococci. Penicillin solution of different concentrations which are placed in the cylinders diffuse in the agar causing zones of inhibition of bacterial growth. The size of the circular zone bears a relationship to the amount of penicillin present.

(e) & (f) Morphological changes exhibited by bacteria grown under penicillin.

(e) normal streptococci.

(f) Penicillin treated streptococci have poorly formed cell walls. The cells therefore subjected to osmotic gradient of the outside medium start swelling.

9

Wonder Drugs From Soil Moulds

THE SOIL is full of teeming millions of microbes. The bacteria and the spores of fungi might take a sojourn from their home and travel in the air around us. It was one such spore of *Penicillium notatum* that sneaked into the Petri dish which Fleming was observing.

Scientists interested in soil fertility and eradication of plant diseases have been studying the microbial population of the soil for years. One such scientist, Selman A. Waksman had been working on problems concerning distribution of microbes in the soil. Waksman was interested, from his early scientific days, in certain small colonies of microbes that developed from the soil in Petri dish culture. Seen with the naked eye, they looked somewhat like bacteria, but under the microscope had the appearance of fungi. His teachers could not help him to identify them. After much intensive study he came to the conclusion that they were the representatives of what was then an obscure group of microbes called actinomycetes or ray fungi, so called because of the characteristic radiating pattern of structure as seen under the microscope.

Waksman was born in a small town called Novaia-Priluka in the Russian Ukraine in a primitive thatched house. He was born at the time of the Czars and the revolution was in the making. Waksman's family was Jewish and the Jews were in a state of uneasy truce with the rest of the population. By the age of 20, he had lost his father and mother and, after somehow completing his school education, persuaded by his cousin who had settled in the USA, he left Europe in the year 1910 for the USA, at the age of 22. On the advice of his cousin, he came into contact with another Russian emigrant, Dr. Jacob Lipman, head of the Department of Bacteriology of the Rutgers College, New Brunswick. He discussed with him his plans for future education and after having obtained a merit scholarship he began his scientific career as an agricultural student at Rutgers in 1911. He graduated with a Bachelor of Science degree in 1915 and then went on to do some outstanding work in the field of soil microbiology and in understanding the new group of microbes—actinomycetes or the ray fungi.

Around 1939, Waksman and his group of workers turned their attention to anti-bacterial substances produced by the ray fungi. In 1940, Waksman isolated an active substance called actinomycin from one of the actinomycetes. But unfortunately, this proved toxic to animals and he, therefore, continued to examine systematically other varieties of actinomycetes as well as countless other types of microorganisms. To understand the tediousness of the process that Waksman and his co-workers adopted to isolate a potent antibiotic, let us learn ourselves the methods they followed.

Screening Microbes for Antibiotics

Soils from various regions were procured and after air drying were taken as 1 or 10 g samples and dissolved in a known amount of steam-sterilized and cooled water in conical flasks. The suspension was diluted, step-wise, to give the dilutions of 10, 100, 1000, 10,000 or 1,00,000 times or even more. A small amount of the sample from the desired dilution was placed in a Petri dish over which was poured and uniformly spread the appropriate nutrient agar. Colonies of actinomycetes, bacteria and fungi would develop depending on the type of food base in the agar media. Each of the colony was then isolated on to agar in test tubes containing specific media. They were then identified, labelled and kept as stock cultures. Each one of those organisms was then tested for anti-bacterial activity.

On the surface of nutrient agar is streaked with a sterile needle the organism to be tested. After it has initiated some growth the disease-causing bacteria are streaked perpendicular to the original streak. Depending on the anti-bacterial nature of the test organism, the bacterium may or may not show growth inhibition. By this streak test, mass screening of the various organisms for powerful antibiotics can be done. After thousands of organisms have been tested one may come across an antibiotic producer. This must then be further tested for the potency of antibiotic action over a wide range of disease-producing bacteria. After this the chemical purification is attempted and the chemically pure substance will be tested clinically on laboratory animals infected by bacteria. The newly isolated substance may be a powerful antibiotic and cure the mice of infection but may have a severe toxic side effect. In such an event it has to be discarded. Actinomycin, mentioned earlier, is one such example. After such tedious experiments the most promising ones are tested against pathogenic organisms in man. After repeated trials and confirmation in clinical trials, the antibiotic is approved for production on mass scale and released commercially.

Waksman and his assistants had to face numerous disappointments; isolation of actinomycin in 1940 and streptothricin in 1942, both of which had many limitations and could not be used clinically, are examples. Their efforts were, however, rewarded in 1943 when streptomycin was extracted from *Actinomyces griseus* (then called *Streptomyces griseus*).

By the time streptomycin was isolated a total of about one hundred thousand different cultures of various types had been examined or screened.

Although streptomycin is mildly toxic after isolation, it was soon apparent that a discovery had been made of similar importance to that of penicillin and, further, that this drug was complementary to penicillin—a powerful weapon against many microbes that could not be killed by penicillin.

Streptomycin

Streptomycin occupies a unique position among the antibiotics because of its preferential action on gram-negative and acid-fast bacteria. The chief merit of streptomycin lies in its ability to attack those bacteria that are not affected by penicillin. It has proved useful in the treatment of plague, meningitis due to *Haemophilus influenzae,* and infections by the *Salmonella* group and *Klebsiella pneumoniae.* It has been also useful in treatment of dysentery caused by *Shigella.* The drug has proved to be a boon in the treatment of early progressive infiltrating pulmonary tuberculosis. The discovery of streptomycin and its powerful effect against the tubercule bacilli was a great therapeutic advance, but it was soon realised when used by itself that it was of limited value as the organism was developing resistance to the drug. While Waksman was at work on soil fungi, other scientists were experimenting with various chemical substances and testing them by the trial-and-error method against tubercule bacilli. In 1941, Bernheim found that para aminosalicylic acid was effective. Again it was found that by itself this substance was found to give an initial improvement and soon the organisms tolerated this drug also

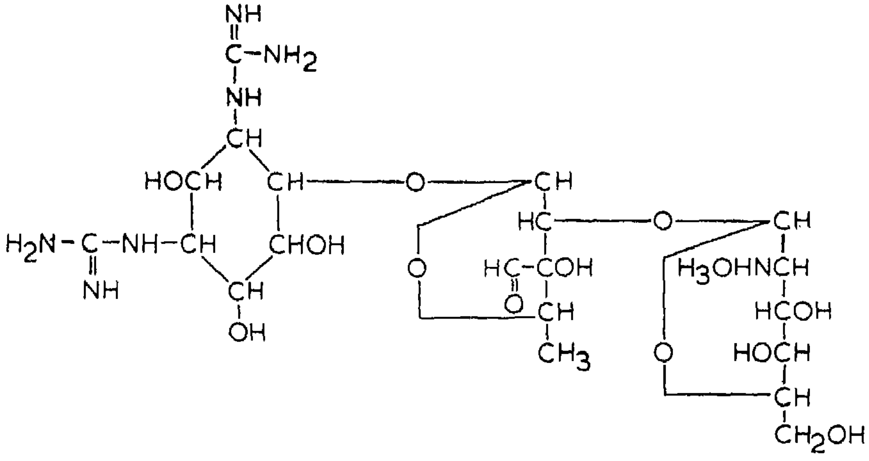

Fig. 6 Streptomycin made up of three moieties:
(left to right) Streptidine, Streptose and N-methyl-l-glucosamine

However, when streptomycin was administered along with para aminosalicylic acid, streptomycin retained its therapeutic value for a long time and thus a judicious combination of the two drugs could annihilate the tubercular bacteria. In addition to the above drugs, another chemical, isonicotinic acid hydrazide (described already), when administered with either of the above two, has a salubrious effect in the therapy of tuberculosis; and the therapy has to be on a long-time basis. Thus, streptomycin has come to be the main stay in the treatment of this dreaded disease of mankind.

Streptomycin is a condensation product of three compounds, Streptidine, Streptose and N-methly-l-glucosamine. A slight change in the streptose group gives rise to a carbinol group and yields the medically useful drug dehydro-streptomycin.

Several types of action mechanism have been postulated for streptomycin and controversy exists concerning the major site of action in the microbe that produces the lethal effect. In the initial stages streptomycin appears to interfere with certain active centres of the bacterial cell membrane, and once it has diffused into the cell the site of action appears to be the ribosomes. It prevents the attachment of messenger RNA to the specific sites in the ribosome and thereby inhibits protein synthesis.

Antibiotics from Other Actinomycetes

With the success obtained with streptomycin, microbiologists started screening all the organisms coming under the group, actinomycetes, for antibiotics, and the result is the successful introduction of quite a few antibiotics isolated from many other species of *Streptomyces* (now called Actinomyces). A

Chloramphenicol (Chloromycetin)

The antibiotic property of chloramphenicol was first described by P. Ehrlich (not to be mistaken with the "Father of Chemotherapy)" in the year 1947. Originally isolated from the organism *Streptomyces venezuelae,* it has a relatively simple structure and is now marketed as the synthetic product.

Chloramphenicol is effective against a wide range of organisms. It is active against a number of gram-negative and gram-positive bacteria and certain rickettsial infections. It is particularly useful in the treatment of typhoid fever caused by *Salmonella typhae* or *Salmonella paratyphi* A or B, infections which are notably resistant to all other antibiotics and chemotherapeutants.

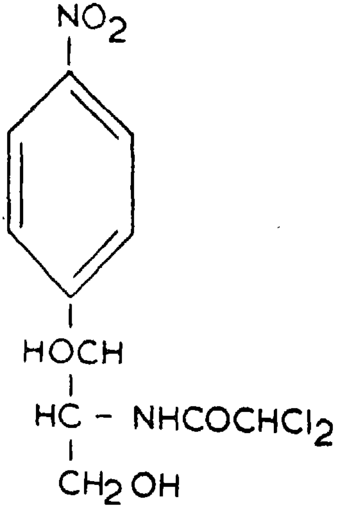

Fig. 7 Chloramphenicol

Chlorampenicol has been shown to block protein synthesis in bacteria. The site of inhibition has been shown to involve the transfer of amino acids from the transfer RNA component of RNA to the peptide bond forming site in the ribosome.

Tetracyclines

A group of compounds coming under this head have Tetracycline as the parent compound and the name being derived from the four rings of their chemical structure. Chlortetracycline (aureomycin) and its derivative demethylchlortetracycline (declomycin) substitute a chlorine atom in the basic structure and oxytetracycline (terramycin) possesses an hydroxyl group in the ring.

Chlortetracycline is a golden yellow compound isolated from *Streptomyces aureofaciens* by Duggar in 1948. Its activity appears to be about equal that of penicillin for the gram-positive cocci and to that of streptomycin for the gram-negative bacilli. Dozens of other tetracycline antibiotic have been synthesised to get maximum therapeutic effect and minimal of toxic effects.

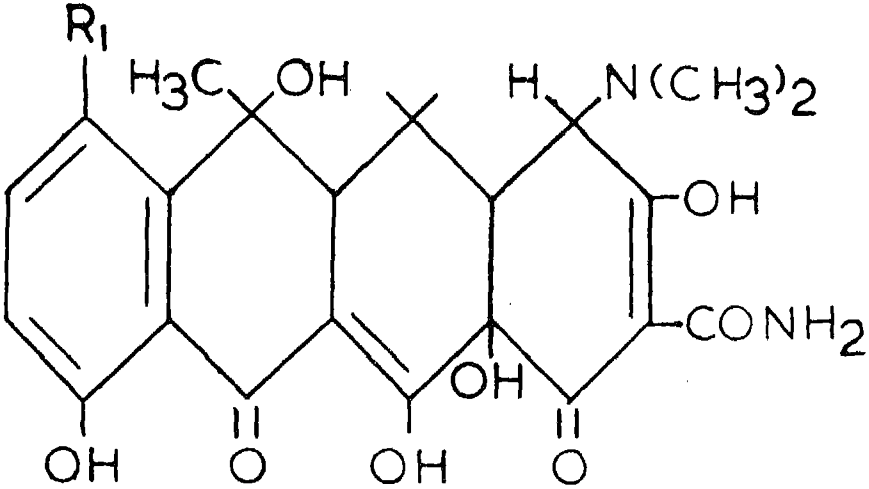

Fig. 8 Tetracycline

Initial investigations with microorganisms have shown that tetracyclines also interfere with protein synthesis, by preventing the incorporation of aminoacids to form the peptide bond. Tetracyclines have also the avidity to bind metals, called as chelation. How far this property interferes with the other vital aspects remains to be clarified.

Antibiotic Therapy of Mycoses

While all the antibiotics described till now are curative for diseases of bacterial origin, some of the deep and superficial infections caused by fungi were without any potent therapeutant till recently. One of the first antifungal antibiotic discovered is *griseofulvin* which is commercially available as *griseovin.* Although isolated in 1938, and its pronounced antibiotic effect was known, it was not tried in fungal infections on man till 1958.

This substance originally isolated from the mould *Penicillium griseofulvum* (this fungus belongs to the same genus as the mould from which penicillin has been isolated) is known to be produced by certain other species of *Penicillium.* The drug when taken orally appears to be absorbed well and acts on the superficial mycotic infection like ringworm, and other dermatophytic infections and by its fungistatic action removes the infection. External application over the infection spot has no effect. This antibiotic has no therapeutic effect on deep mycoses and internal infections. Taken in excess and as prolonged dosage it may produce mild toxic side effects on the patient.

Two more antibiotics more promising than griseofulvin in curing infections caused by pathogenic fungi are Nystatin and Amphotericin B. Both the substances are derived from the ray fungus; Nystatin from *Streptomyces noursei* and Amphotericin B (also called Fungizone) from *Streptomyces nodosus.*

Nystatin is useful in infections caused by *Candida albicans* especially in those local infections involving the skin and mucous membranes such as thrush. It is often given with a broad spectrum antibiotic to suppress the growth of *Candida,* and other yeast-like fungi which may gain an upper hand in the intestines, as the antibiotic in addition to removing the harmful bacteria may wipe out the beneficial bacterial flora of the intestines. Amphotericin B must be administered intra-venously. It is effective against various systemic mycoses such as cryptococcosis, blastomycosis, histoplasmosis and coccidioidomycosis. Although it is a potent drug its use is frequently attended with troublesome but not prohibitive side effects; the initial administration of the drug is accompanied by chill-fever reaction, followed by nephrotoxicity and certain electrolyte imbalances, if the treatment is prolonged.

These drugs have no effect on bacteria. The protoplasmic membrane of the fungal hypha appears to be the site of action. Nystatin somehow produces leakage of intracellular constituents and the drug is bound by protoplasts or membrane containing particles of sensitive organisms. Evidence shows that the binding site of the membrane contains a sterol. Sterols are not found in bacterial membrane and that seems to account for the selective antifungal action of the two antibiotics described above.

10

Whither Antibiotic Therapy?

THE YOUNGER GENERATION OF PEOPLE born in the post-World War II era (after 1945) may not quite realise the magnitude of the problem faced by doctors when treating their parents and grandparents for infectious diseases before the advent of antibiotics. Today, thanks to the outstanding scientific contribution of the older generation, it is taken for granted that most disease-producing microbes can be eradicated by one drug or another. Before 1945, medical wards in hospitals always had a large number of young people seriously ill with diseases such as pneumonia, meningitis, septicaemia, while the post-operation wards were filled with patients having pus oozing out of abscesses, carbuncles and improper healing of surgical wounds. Now, all this is changed due to the discovery of sulphanilamides, penicillin, streptomycin and a large array of antibiotics discovered in recent years. The advent of sulphanilamide drugs and antibiotics brought with it the promise that bacterial diseases might be brought under control, but that promise has not been fulfilled. Although many infections respond dramatically to chemotherapy, tuberculosis, dysentery and typhoid fever continue to be endemic in many parts of the world, while cholera and plague erupt periodically; staphylococcal infection persists in the most advanced hospitals. One major reason for this is that the microorganisms have developed resistance to drugs.

When penicillin came on the scene it could easily eradicate staphylococci. However, by the mid-1950's as many as 90 per cent of staphylococal infections seen in hospital wards were associated with penicillin-resistant strains. Chloramphenicol and erythromycin were fortunately discovered in time to deal with the penicillin-resistant staphylococci and now, where strains resistant to all the three antibiotics are appearing, other synthetic derivatives of penicillin have been produced to deal with them.

Similarly, in the beginning, all gonococal infection could be cured with penicillin. In recent years gonococci resistant to penicillin have appeared and newer drugs have to be used.

Thus an endless race is going on with the microbes on one side, throwing out newer and newer mutants that can overcome all known drugs, and the scientists on the other side, discovering newer and newer antibiotics to combat the new strains. Where all this will lead us to at the turn of this century, is anybody's guess.

The discovery of streptomycin and its powerful effect against the tuberculosis organism was a great therapeutic advance; but, it was soon realised that there were limitations.

When first given to the patient the effect on the disease was remarkable; patients' general condition improved, and X-ray of the lungs showed surprisingly rapid clearance of the foci of infection. But after some weeks, it was found that streptomycin began to lose its effect and finally became useless. The reason for this was the bacilli causing the disease in some way adapt themselves to the drug and have become resistant to streptomycin. But in the case of streptomycin a way was found to overcome this difficulty. When streptomycin is administered with para-aminosalicylic acid, another anti-tubercular chemical discovered around the time streptomycin was discovered, the bacteria did not develop resistance to streptomycin, and prolonged therapy which is essential with this drug became possible to completely eradicate the germ from the infectious patches in the lung.

Mechanism of Resistance

It was believed until very recently that prolonged or frequent use of antibiotics produced an adaptation to the drug by certain bacteria and this change was transformed genetically, and this spontaneous mutation was considered responsible for drug-resistance. As a consequence, a school of thought developed which believed that constant and frequent use of antibiotics will induce too many mutants that are drug-resistant to be thrown out, and, as such, indiscriminate use of antibiotics portends future hazard. However, very recent work on durg-resistance has shown very clearly that this resistance is due to a more dangerous phenomenon at work and is not due to adaptive mutation to drug action, as originally thought of.

If an antibacterial drug is added to a liquid culture of a bacterium that is sensitive to the drug, after a while all the cells in the culture are found to be drug-resistant. Once it was thought that the drug must have somehow induced resistance. What has actually happened, of course, is that a few cells in the original culture were already resistant; these cells survived and the entire progeny of growth seen belatedly comes from these few resistant cells after the majority of the sensitive cells have perished due to drug action. The resistance was not induced by the drug but was already present as a result of spontaneous mutation. Bacteria, like higher organisms have chromosomes incorporating genetic material. From time to time a gene—perhaps one controlling drug-resistance —undergoes a mutation. The mutation of a drug-sensitive gene occurs only once in 10 million cell divisions, and when it occurs it alters a cell's sensitivity to one particular drug or perhaps two related drugs.

For example, in 1955, a Japanese traveller arrived from Hong Kong with a severe case of dysentery. The causal agent on isolation was found to be the typical bacillus dysentery organism *Shigella*. This particular *Shigella* was resistant to sulphanilamide, streptomycin, chloramphenicol and tetracycline. In the next few years the incidence of multiple-drug-resistant shigellae in Japan increased and there was a number of epidemics of severe dysentery.

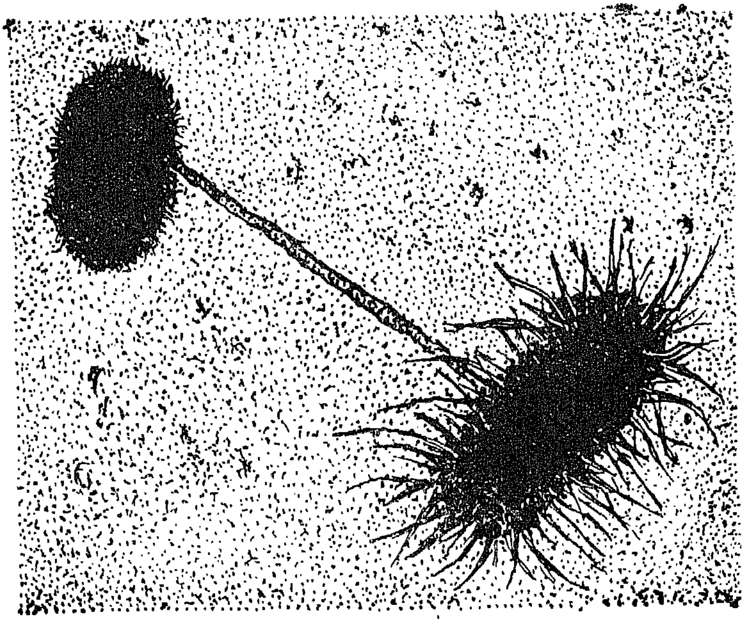

Fig. 9 Multiple-drug-resistance factor 'R' being transferred from one bacterium to another by conjugation (diagrammatic representation).

This severe outbreak of epidemic could not be explained solely on the basis of spontaneous mutation alone, for the following reasons: Both drug-resistant and susceptible shigellae could be isolated from the same patient. Again, administration of a single drug, say chloramphenicol, to patients harbouring sensitive organisms could not cause them to excrete shigella that are resistant to four drugs. Then it was found that many of the patients harboured the normal colon bacteria, *Escherichia coli*, that had become resistant to the four drugs. This is very likely, as these patients might have been administered successively, at different times, the four antibiotics for therapy of different diseases they might have contracted. Now it has been shown that the resistance to four drugs has been transferred in one step from the colon bacteria to the shigella within the digestive system. This transfer might take place by three known mechanisms of genetic transfer. One is transformation, which is a form of genetic transmission in which the hereditary material DNA extracted or excreted from a donor cell enters a recipient cell and is incorporated into its chromosome. In this way a mutated gene controlling resistance to a drug may be transferred to a drug-sensitive cell. The other method is 'transduction' where the transfer of the gene material from one bacterium to another is mediated through bacterial viruses. The third method is the transfer of DNA by sexual mating or conjugation. The last method has been experimentally demonstrated in bacteria. In the case of bacteria, multiple-drug-resistance could be identified extrachromosomally as the R factor and this can be transferred by conjugation. Such conjugation can take place not only between the male and female of the same species, but also between the female and female of the species and even between two genera as between *Escherichia coli* and *Shigella*. Japanese scientists have shown that the resistance factor called "R factor" can be transferred from *Proteus* to *Salmonella* which causes typhoid fever, to *Vibrio cholerae*, the agent for cholera, to the plague bacillus *Pasteurella pestis*, and so on. Because of the rapid transfer of the drug-resistance factor from one bacteria to another this is called as 'infectious drug-resistance'.

This newly discovered drug-resistance is likely to pose a great public health threat throughout the world and the range of bacterial hosts acquiring multiple-drug-resistance are bound to increase.

We must assume that additional drug-resistance factor will appear and proliferate as new antibiotics come into use.

The indiscriminate use of antibiotics for curing all and sundry diseases produces in our body a proliferation of the multiple-drug-resistant but harmless *E. coli* bacteria of the digestive system, which will pass on the R factors to the susceptible disease-producing bacteria, for eradication of which the antibiotic in question has been given. Unless we put a halt to the prodigal and indiscriminate use of antibiotics and synthetic drugs, we may soon be forced into the pre-antibiotic era of medicine.

11

Immunity

MANY OF THE CHEMOTHERAPEUTANTS and antibiotics by themselves cannot completely remove infectious germs from our body but for the fact that our own body is, silently and without much ado, helping these drugs by creating conditions in the body unfavourable for the existence of the invading microbes. If people survived many of the worst afflictions due to microbes in the pre-chemotherapeutic era, it was because of the capacity of the living body to produce certain defence barriers against the invader, which persists long after the disease and wards off a second attack. In fact, Thucydides, a contemporary of Hippocrates, did make the following observations about the plague of Athens even as early as 430 B.C. although no one knew the cause and the contagious nature of the disease. "No one was ever attacked a second time or at least not with fatal result."

Nearly two thousand years later, in the last decade of the eighteenth century, Edward Jenner of England observed the very phenomenon and discovered vaccination against smallpox.

Edward Jenner was born in 1749 in a village called Berkeley in Gloucestershire, England. In his youth Jenner himself underwent an inoculation against smallpox. This method of inoculation, which was introduced into England early in the eighteenth century by Lady Mary Wortley Montagu, should not be confused with vaccination.

When Lady Mary brought back a knowledge of this practice from the Arabian countries, it was already realised that one attack of smallpox usually conferred protection against a second attack (immunity). People were, therefore, inoculated with the poison from a mild case in the hope that they too would not suffer severely from the disease. What was then not appreciated was the fact that a mild attack of the disease in one case might give rise to a serious form in another patient. Many deaths occurred amongst inoculated people and finally in 1840, inoculation was forbidden by law. Young Jenner himself underwent this inoculation, a process that was both an unpleasant and a horrible experience. Jenner never forgot this and when he later took to medical studies he was much interested in smallpox. He was also aware of a belief, considered by many medical men at that time as being mere superstition, that Cowpox, a mild disease, which produced a spotty eruption of the affected parts, protected people against smallpox.

On the completion of his studies, luckily for mankind, Jenner opted for practice in his native village instead of a lucrative partnership for practice in London itself with a well known medical man.

Infinite patience was one of Jenner's outstanding characteristics. For many years he visited the farms, made friends with the dairy maids and others working with cows and then kept a careful record of all cases of human Cowpox he could find. He came to the conclusion that the proverbial apple cheeks of dairy maids, so few of whom were scared by smallpox marks, must be due to the fact that these girls escaped the disease because many of them at one time or another had suffered from a slight spotty eruption on the hands and arms contracted while they were handling cows with Cowpox infection.

Jenner decided to test his hypothesis but it was a crucial one. In the year 1796, Jenner took some pus from Cowpox eruptions in the hand of a milkmaid called Sarrah Nelmes and inserted it by two superficial incisions into the arm of James Phipps, an eight year old boy. This was the first vaccination in history. Two months later, virulent smallpox matter was injected by Jenner into the boy's arm. Imagine how Jenner must have felt, if he had failed in the crucial test and the boy had died of the virulent infection! But Jenner's astute observation was amply rewarded. The boy did not contract smallpox and remained completely unaffected by the injection. The boy had acquired immunity to smallpox.

There were many men, both in the medical profession and outside, who at first opposed Jenner's vaccination; but England soon realised the importance of his discovery to the country, nay, to mankind as a whole. Another weapon against disease had been added to the increasing list of armouries: vaccination or active immunization.

We have come a long way from Jenner's time on the road to immunity and the horizons are widening day by day.

Acquired Immunity

When any foreign organism is introduced into your body, the body is induced to produce certain substances which knock off the invader. The invader in question is the *antigen* and the substance formed against the antigen is the *antibody*. But a particular antibody formed against a particular antigen can inactivate only that antigen and none else. In other words, antigen-antibody reactions are specific. But fortunately for Jenner, he was producing in the young boy the antibody to Cowpox which could inactivate the closely related smallpox organism. If the antibody to Cowpox was only active against the Cowpox organism, Jenner could not have got away with his vaccination experiments and the boy would have died of the smallpox injection. But fortune favours not only the brave but also the trained mind!

Acquired immunity is specific and is usually subdivided into two main groups, active and passive. Active immunity refers to specific immunity built up by an individual in response to an invading organism. Such immunity can be obtained either in a natural manner—naturally acquired active immunity—or as the result of deliberate immunization with a vaccine—artificially acquired active immunity. The observation that a person surviving a smallpox infection does not get it for a second time exemplifies the first category. The fact that Jenner could produce immunity in the boy to smallpox by artificially vaccinating him with a related and less virulent Cowpox organism exemplifies the second category of artificially acquired immunity. In both instances the organism itself or a less virulent or closely related organism induces the body to produce the antibodies against the pathogen.

Passive immunity refers to the protection afforded by the introduction into one individual of antibodies produced in the blood of another individual or animal that has been actively immunized. Ready made antibodies are introduced into the body and the body need not manufacture them. This type of immunity can occur under natural conditions—naturally *acquired passive immunity*—and is exemplified by the resistance to some common infections of new born babies who have received some of their mothers' antibodies when inside the womb. Artificially passive immunity refers to the type of protection given when the antibodies in the serum of an immune person or animal are injected into the body of the patient.

12

The Basis of Immunity —Antigens and Antibodies

What Are Antigens?

TODAY the technique of immunization provides protection against all the significant diseases that have not been eliminated by public health measures or by therapy with chemicals or antibiotics. Although the practical problems of immunization have been solved, immunology remains an important branch of medicine.

Soon after the 'germ theory' of disease, propounded by Louis Pasteur and confirmed by Robert Koch, came into prominence, Roux, a student of Pasteur, working with diphtheria-infected mice, observed that the infection focus was localised in the throat but toxic symptoms were manifest in the internal organs like the liver, spleen and stomach of the animal. He visualised that death was due to translocated metabolites produced by the diphtheria organisms and called this microbial poison or toxin. This was later confirmed in other bacterial diseases and it is now clear that the pathogenic (killing) potential of infectious organisms is related to their potential to produce toxins. If the toxins are produced by the bacterium and secreted into the environment of the body or the medium they are called exotoxins. But if the toxins are bound inside the bacterial cell and released only after the death (autolysis) of the bacterium they are called endotoxins. Toxins have been identified with many disease-causing bacteria, and in most cases they are macromolecules, proteins or polysaccharides, and they are antigenic. When injected in small doses they confer resistance to the animals not only against the toxins but also against the bacterium.

Not only a bacterium and its toxins are antigenic; any macromolecular substance which is alien to our body when injected, will behave as an antigen. (As a general rule, components of an individual's own tissues do not act as antigens in the body.) Antigens must have a molecular weight of at least 10,000. Proteins are the most common antigens, but some are more antigenic than others; ring structure and acid groups in the molecule are essential. Certain polysaccharides are also antigenic. Lipids, by combining with proteins or polysaccharides, become antigenic. Many foreign substances of small molecular size cannot act as antigens alone but link with protein to form antigens. Such substances are called haptenes. The specific character of the antigen formed is dependent in such cases on the haptene, and the antibody reacts with it.

In spite of the great similarity in chemical structure of the forms of life, the proteins of each species are different and highly specific. The basic units, the amino acids, are the same but the relative quantity of each and the order and manner in which they are linked together to form the giant protein molecules vary infinitely. In this perspective complex carbohydrates and lipids are, however, generally less highly specific in plants and animals but not in microbes. Even without these, however, the specific nature of proteins would mean that man and higher animals produce antibodies whenever the living material (which always contains protein) of another species infects their tissues. There are, in fact, differences even within the species and among closely related individuals.

Micro-organisms are no more antigens than any other source of foreign material, but they act as antigens more frequently because they often invade the living tissues. Food proteins, for example, would be antigenic, if injected, but taken through the alimentary tract they are digested and enter the blood streams not as proteins but as amino acids which are not foreign at all.

A bacterial cell may contain or produce many substances capable of acting as antigens. They are:

1. Cellular or somatic antigens. These are components of cell substances of the microbe. They are sometimes referred to as O antigens.
2. Surface antigens. Compounds restricted to the outer layer of the cell.
3. Capsular antigens.
4. Flagellar antigens—called H antigens.
5. Exotoxins.

Antibodies and How They are Formed

Dr. Nossal, Deputy Director of Immunology of the Walter and Eliza Hall Institute of Medical Research in Australia, compares conceptually the production of antibodies similar to a large industrial organization that manufactures a wide range of products in many different factories. First the factories, that is the cells that make antibodies, then the products, the antibodies, and then finally specification-presenting customers, the antigen molecules.

Astrid Fagraeus of Sweden suggested that the actual producers of antibodies were a family of specialised cells called plasma cells, which show up in considerable numbers at the site of infection, as inflammation gets underway. She found that two days or so after she had injected a vaccine intravenously into an experimental animal, 'plasmablasts' began to appear in its spleen. These young plasma cells divide rapidly, and after a few days their progeny become more specialised; the nucleus of the cell shrinks and the surrounding cytoplasm expands. The fact that this cytoplasm is rich in ribonucleic acid (RNA) which directs the synthesis of protein, was taken as a strong indication that the plasma cells actively produced protein—that is to say, an antibody. Albert Coon at Harvard Medical School confirmed this by showing that plasma cells do produce an antibody. Nossal and his co-workers have further confirmed this beautifully by simple experiments. They selected for their work the typhoid producing bacterium *Salmonella typhi* which has flagella for movement and the protein extract of the flagella served as antigen. Antibody to the flagellar protein was prepared by injecting the flagellar protein into experimental rabbits. The idea was that when the antibody containing serum or antiserum, when tested on the live typhoid bacillus, will fix the flagella and therefore paralyze and immobilize its movements.

To test the hypothesis that antibody is produced in the plasma cells after sufficient incubation period, the lymph node of the rat receiving the infection of antigens was removed and the individual cells were teased out. Each cell was placed in a drop of tissue culture liquid as a hanging drop on the underside of a coverslip. The cell was incubated for several hours at 37°C. All this was done under sterile conditions. Having given the cells enough time about 10 *Salmonella* bacteria were introduced into the drop and observed under a microscope.

If the bacteria were of the same strain as the one from which the flagellar protein, or antigen was extracted, they stop moving promptly within a few seconds. On the other hand, bacteria of any other strain will go on swimming indefinitely. The action is so specific that the antibody that rapidly immobilizes *Salmonella typhi*, the cause of typhoid fever, has no effect on *Salmonella paratyphi*, the cause of paratyphoid.

Of the many different cells in the teased out preparation of the lymph, only the plasma cells showed antibody activity. The other cells, like lymphocytes and scavenger cells, give no evidence of antibody production. The mature plasma cells have a large amount of antibody and these are formed five days after contact with antigen. The immature cells, called plasmablasts, on the first and second day have very little antibody, as they are cells where the manufacturing is at the highest peak and the products of manufacture—antibody—are not yet ready.

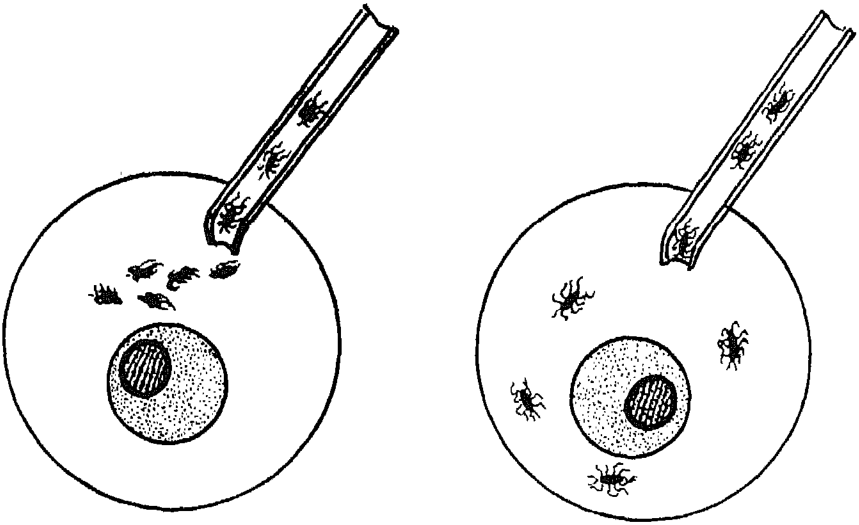

Fig. 10 Flagellar protein of *Salmonella typhi* was injected into rat. The antibody producing cells were isolated. When *Salmonella typhi* were put in contact with the cells (left) they were immobilized. *Salmonella paratyphi* bacilli, a related strain, were unaffected (right).

It was also shown by Nossal and his co-workers that each plasma cell produces a specific antibody. Or, in other words, the different plasma cells in the lymph were busy making different antibodies and thus there was a division of labour; the prevailing principle being one cell, one antibody.

The main outline of the production is as follows. As the order for an antibody comes in, by way of arrival of an antigen, the white cell construction centre (the lymph nodes, spleen, bone marrow, as the case may be) commences to turn out into miniature factories that will produce the antibody. These units start as immature plasmablasts and take a little time—matter of days—to tool up for full production as mature plasma cells; the end line of the assembly that contains maximum antibodies.

The next question that will be asked is: what accounts for the great speeding up of antibody production that occurs in a person who had already been vaccinated or has recovered from an infection?

'Immunological memory', as it is called, is by all account the most fantastic feature of immune response. The production of antibody in this case is so rapid that the infection is stopped in its track. This is achieved by a double-pronged drive. Greater number of plasma cells are produced and greater number of antibodies are produced in each cell.

The Lock and Key

Antibodies themselves are the manufactured product at the end assembly line of the factory. They are chemically a portion of the blood serum called globulin or globular proteins and the average weight of each antibody molecule is 160,000 (10,000 times the weight of oxygen atoms). The sites of chemical activity in the antibody molecule which binds the 'invader' (antigen), however, are represented by a relatively small portion of this complex molecule. A single site may be visualised to be as equivalent to the region occupied by three to five of the hundreds of amino acids that go to make up an average antigen, or an equally small number of monosaccharide

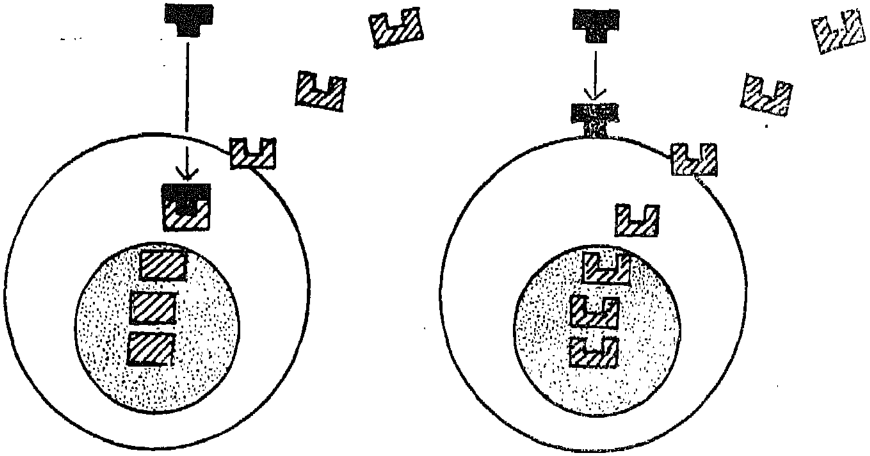

Fig. 11 Two theories on antibody formation is shown. The antigen enters a plasma cell and forms a template from which a complementary antibody is produced (left). The other theory suggests that the mere contact of antigen and a given plasma cell signals DNA in the cell nucleus to start directing production of antibody (right).

unit in a polysaccharide. While these small regions of active union on the antigens are called antigenic determinants, those on the antibody are called specific patches. According to the classical theory, the active site or the determinant, in the antigen combines with the patch in the antibody, because the two are complementary to each other like a lock and key.

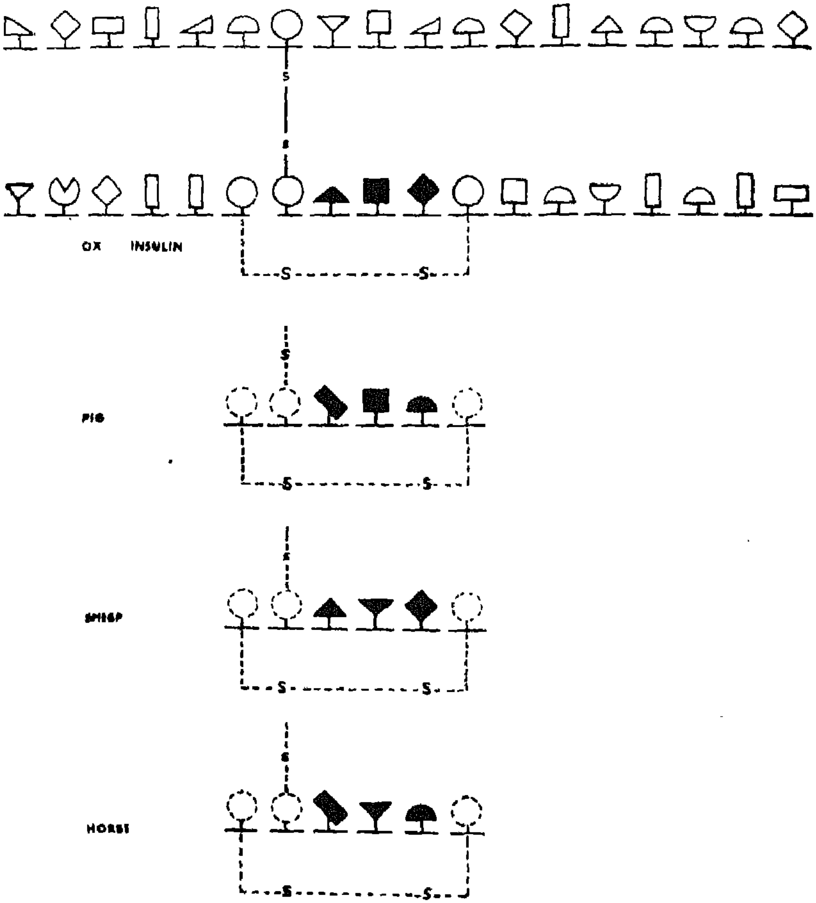

Fig. 12 Insulin molecules produced by different mammals are slightly different from each other. Sequence of amino acid units in the ox insulin is at the top. Only three units (dark) differ from those found in pig, sheep and horse. Insulin of man is exactly identical to that of pig. Insulin of sheep, horse and ox may be antigenic to man.

Once the active site of the antigen is bound, it is no more reactive and is immobilized or knocked out of action.

We have as yet no proof as to how exactly the formation of antibody is triggered off; whether the antibody is moulded on the antigen so that they fit into each other or the antibody is synthesised by remote control. The former would imply the entry of antigen molecule into the antibody producing cell (plasma cells) and the latter implies that the antigen need not enter the cell but somehow encourage production in the lymphatic nodes and other regions of antibody synthesis, the type of plasma cells which are, as it were, 'born' with the capacity to produce antibody to specification demanded. The antigen stays at the periphery and somehow communicates, as it were, by remote control. The body is equipped with a variety of clones of cells of various capacities. Each group of cells has the potential to react to a particular antigen, and the arrival of the antigen simply triggers the already prepared cells to make the appropriate antibody. The plasmablasts first appear to come into surface contact with the antigen and this is sufficient to launch the cells to manufacture antibodies.

The specificity of the antigen and antibody reaction may perhaps be illustrated with an example. Most proteins are antigenic to an organism that has not been concerned in producing them. At present, the structure of one protein is well known to compare its chemical structure and immunological activity. This is insulin, one of the smallest proteins produced in our body, which converts the excess sugar in our body into a form in which it is stored, glycogen. Insulin is produced by the Islets of Lagerhanes of the pancreas and when this is insufficiently produced or not produced, excessive sugar accumulates in the blood and the danger is signalled by sugar in the urine. External source of insulin is injected into such patients to keep the blood-sugar level normal. Of course, insulin is not antigenic in the animal that produces it, and it also happens that it is a mild antigen. Most diabetics can receive beef or sheep insulin for some years without any trouble. Some people, however, become resistant to insulin because they are making antibodies against it. This is because of the 51 amino acid units in a single insulin molecule, 48 have the same arrangement in the insulin of different species; the sequence of units in one segment of three units, varies. If insulin from beef or sheep is antigenic to some people it is because the human body recognizes the difference in the sequence of three amino acids as different from its own insulin, which it has now stopped producing. However, pig's insulin does not produce antibodies in man as the sequence here is completely identical to that in man!

Even though insulin is a poor antigen, it still presents rather clearly an important question; How does an insulin-resistant diabetic 'recognize' the tiny difference between beef or sheep insulin and his own insulin, and so make antibody against the former? This is the most astounding feature of the living body and scientists are working throughout the world to unravel this remarkable phenomenon of the fight of bodies against foreign germs or proteins.

Antigen-Antibody Reaction: A Visual Demonstration

Antigen-antibody reaction may be visually demonstrated by bringing the liquid phase of the antigen and antibody together and after incubation, precipitin rings are formed. When these experiments are performed in a solid agar media by placing the antigen and antibody in two adjacent wells dug out in the agar, clear precipitin bands are formed at the place of contact of antigen and antibody diffusing into the solid media from the wells. Such antibodies are called precipitins. This method is called Ouchterlony double diffusion method of demonstrating antigen-antibody reaction. In the presence of electrolytes (saline) antibody may also bring out a sticking together of, or clumping of microbial cells. The formation of clumps of cells is called *agglutination* and the antibodies in question are referred to as *agglutins.*

In the plasma of blood we have a component called a *complement* which appears to be protein in character. Some cells when combined with homologous antibody, are lysed by the complement, but whether lysis occurs or not, complement will unite with an antigen and antibody and is thus fixed or attached. Complement will not unite with either antigen or antibody alone. If there is a known antigen which is incubated with an unknown serum and complement, complement will be fixed only if the specific antibody is present in the serum. Fixation of complement is not, however, a visible reaction in most cases, and this is tested by using 'indicators'.

If we mix two clear liquids of hydrochloric acid and sodium hydroxide no visible change occurs. In order to find if the acid is neutralised we add an indicator; a substance that undergoes a visible change in the presence of a free acid.

Similarly, an indicator is added here to tell us by visible change the presence of the free complement. The indicator is another antigen and its antibody—red blood cells of sheep (as antigen) and their antiserum (as antibody) are added as indicator. The complement, if it has not already acted with the first antigen-antibody, is now free to act with the indicator antigen-antibody, sheep erythrocytes and its antiserum, and lyse it. This reaction is called *haemolysis*.

Sometimes heavily capsulated bacteria, like the bacteria causing pneumonia, *Diplococcus pneumoniae*, show a distinct swelling of the capsule when combined with specific antisera. This is known as *capsular swelling* and used as a test for antigen-antibody reaction.

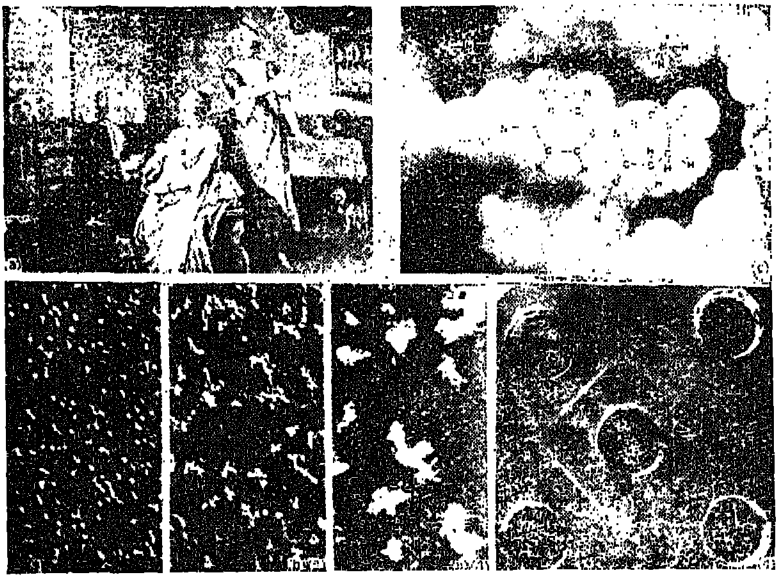

Plate 6. (a) Edward Jenner vaccinating a child with the pus from the cowpox infected spot from the hand of a dairy mad, Sarah Nelmes.

(b) What Jenner did not know then is now known. Picture showing a virus causing a disease in birds, being precipitated by its antibody: (1) virus particles in plasma, (2) aggregation of virus particle after one minute exposure to antiserum, (3) after 3-minute exposure to antiserum; the virus (antigen) has been precipitated by antibodies in the antiserum.

(c) Molecular representation of a portion of antigen-antibody showing the chemically reactive sites where they are bound and hence the precipitation.

(d) Visual demonstration of antigen-antibody precipitation by the agar double diffusion method. Central well has antiserum and the peripheral wells different concentrations of antigen. Note the precipitin band at the point of contact of antigen-antibody.

13

Application of Antibody in Therapy and Prevention of Disease

Active Immunization: Microbes as Antigens

HERE let us discuss some of the practical aspects of preparation of substances involved in active immunization to fight diseases of micro-organisms.

The term 'vaccine' is used to refer to antigenic material containing micro-organisms. The preparation of a bacterial vaccine involves many steps:

1. A highly virulent pathogen must be obtained.
2. It is grown in large quantities in synthetic media.
3. The surface growth is removed and suspended in normal saline.
4. It is then killed at a temperature which is just sufficient to kill it, or with chemicals.
5. The number of dead organisms present per unit of the preparation is estimated and the material diluted with normal saline to a concentration that is standard for the particular vaccine.

6. The vaccine must be sterile (not contaminated) and this should be tested.

Living cultures are used where killed cultures are not effective and also if the organism produced no toxin. But here, however, cultures with attenuated (weakened) virulence are used; and also the cultures, to be on the safer side, are introduced into a resistant tissue of the human system rather than the blood vascular system. A specific antiserum to the micro-organism may be injected at the same time as the living organism into the body to remove the injurious effect, if any, produced by the micro-organism.

Active immunization with killed cultures is successfully employed in many bacterial diseases and in some diseases due to the virus and rickettsiae. Vaccines are essentially given as a preventive measure or as prophylaxis.

Usually, a vaccine contains only one species of micro-organism. Occasionally mixed vaccines are used. A vaccine containing paratyphoid A and paratyphoid B as well as typhoid organisms may be used to confer immunity against all the three infections. Such vaccines are called polyvalent vaccines.

Microbial Toxins as Antigens

Microbial poisons secreted by bacteria are referred to as toxins (exotoxins). When bacteria are grown in liquid culture, toxins will accumulate in the liquid and these are mixed with other substances in culture media. Toxins are prepared as follows:

1. Pure culture of a strain of the bacteria known to produce the toxin is grown in sizable container in liquid nutrient medium.

2. The culture after growth is filtered through a bacteria proof filter.
3. The fluid filtered portion is tested for sterility—free from all bacteria.
4. The strength of the toxin is measured by the effect of graded amounts on experimental animals.
5. Appropriate dilutions are done to get the standard unit of a toxin.

The toxin so prepared may be used in the following forms:

a) The toxin as such. This is given in small but repeated doses to avoid severe reaction.
b) The toxin is altered by the addition of chemicals, like formalin, which lessens the side reaction, but not the immune reaction. Such a preparation is called *Toxoid.* The fluid toxoid is referred to as FT.

A fluid toxoid obtained as above can be precipitated by alum (potassium alum); since the precipitate dissolves slowly in the tissues, it acts over a long period of time and fewer injections need be given. Alum precipitated toxoid (APT) is very well in use.

All the above forms, when injected, produce the antibody to the toxin called antitoxin and offer specific immunity against bacteria whose disease-producing capacity (pathogenicity) is solely due to the toxin.

Passive Immunization

The substances discussed till now were antigens which, when injected, activate the production of immune bodies (antibodies) in our body. We shall now discuss the method of introducing into the human body ready-made antibodies for protection against diseases (passive immunization).

Antibody containing sera (immune sera) is obtained from actively immunized horses. The horse is capable of developing antibodies against many infections and can be bled repeatedly for many months. Cows, rabbits and other animals are also used as sources of immune sera.

Preparation of a Typical Antiserum

1. A healthy horse is given repeated injections of exotoxins, or killed or living culture of the micro-organism, as the case may be.
2. When the antibody content of the serum is high the horse is bled in the jugular vein. As much as 4½ litres can be taken out at a time.
3. After allowing the blood to clot, the clear serum is removed.
4. The antibody material in the serum is concentrated and purified by precipitation and dialysis.
5. The sterility of the preparation and the potency are tested before release for use.

Varieties of Antiserum

A serum containing antibodies to killed or live culture of bacterium is *antibacterial serum*. We speak of *antimenningococcic serum, antipneumococcic serum*. If the animal were to be immunized by the exotoxin of a bacterium the serum is antitoxin serum or just antitoxin. Examples are diphtheria antitoxin and tetanus antitoxin. They are also called Anti Diphtheria Serum (ADS) and Anti Tetanus Serum (ATS).

A polyvalent serum is one that contains antibodies against more than one type or species of organisms.

Human Gamma Globulin

Concentrated antibodies of human origin are obtained from blood donation centres. As the antibodies are essentially gamma globulin of the blood serum, the gamma globulin fraction is separated from the pooled blood by precipitation with alcohol at low temperature. Only about 7 cc of a 16% solution can be got from 500 cc of blood. This gamma globulin fraction (GG) contains antibodies against childhood diseases that the adult donors must have experienced in the past. This (GG) is now recommended for the passive immunization of young children against measles. Its use in the protection of persons exposed to jaundice of viral origin (infective hepatitis) is also generally accepted.

Therapy with Serum and Vaccine

Antiserum and vaccine are mainly used as 'protective' against diseases. In a few cases they have been used to cure diseases, *i.e.,* in therapy.

Serum Therapy

Human antiserum is used in treating whooping cough. The serum is prepared from the blood of a person who had the disease and who has been given repeated dose of the antigen to increase the antibody content. Hyperimmune antiserum is taken and the gamma globulin is concentrated and used.

Serum therapy to be effective must be administered as soon as infection is diagnosed. Usually a single large dose is given intramuscularly. Serum recipients may develop hypersensitivity reactions.

Vaccine Therapy

Certain chronic infections such as boils, colitis, may occasionally be treated by the use of vaccine. A mixed vaccine is to be used containing dead organisms of the species most likely to be the cause of the patient's infection. Sometimes, the vaccine is prepared from the bacteria isolated from the patient (autogenous vaccine). Vaccines for therapy are given in small divided doses—the idea is that the antigenic material in the vaccine will stimulate increased production of antibody circulating in blood.

Sometimes vaccines such as typhoid vaccine, or other protein substances are used in what is termed as nonspecific protein therapy. The temporary fever and other physiological reaction that they cause may have a favourable effect on diseases like arthritis and certain allergic conditions.

14

Is Man the Ultimate Victor?

ABOUT A HUNDRED YEARS AGO, Louis Pasteur sighted the enemy, bacteria, and Robert Koch identified it. Another 40 years elapsed before, at the turn of this century, Ehrlich demonstrated the possibility of using chemicals as weapons against man's enemy; chemotherapy was born. But two more decades passed by before practical chemotherapy arrived on the scene; Domagk gave the world Prontosil, in 1934, father of modern sulphanilamide. But a decade later, the most powerful missile to kill the enemy came into use, hailed as the wonder drug; penicillin, a therapeutant offered to mankind by an earthly mould. Since then, for the past quarter of a century, many new antibiotics have been discovered to combat the many bacterial infections of man. For thousands of years man was completely in the dark. Suddenly he has acquired in his armoury, to his own amazement, very powerful weapons with which he hopes to wipe out all traces of diseases that scourge mankind. The question is, will man be the ultimate victor in this battle? Although with the discovery of antibiotics, this appeared possible, it is increasingly realised that man is up against a cunning enemy; drug resistant bacterial strains are fast developing. To eliminate them, newer and powerful antibiotics are being discovered. It is a battle in which no time is lost and no quarter given. Even if one were to assume that all the disease-causing bacteria will be eliminated completely sooner or later with these newer antibiotics, mankind is likely to be scourged by diseases caused by other microbes, especially viruses.

It will be recognised from the accounts given so far that we have drugs against most bacterial infections and some protozoan infections; but none against viral infections. The quintessence of chemotherapy is based on selective action; drugs act on bacterial or protozoan parasites, but not our own body. We are fortunate in that the bacterial wall is chemically different in its constituents from our body and hence is an excellent target for selective action of certain drugs. A few other drug and antibiotics act on certain essential bacterial metabolites, or proteins or nucleic acids and the theory of selective action operates, when a drug is taken in small doses. Fortunately, the dose that is toxic for bacteria is non-toxic for us; continued and heavy doses of these antibiotics do produce side effects in our body.

In the case of virus infections, however, we are dealing with an enemy who is camouflaged; the virus, a nucleoprotein, identifies itself with its counterpart in the host cell and multiplies using the host cell as its base. Any drug that is designed to break up or prevent multiplication of the invading nucleoprotein (virus) is also likely to affect the vital network in the host: the nucleic acid and protein. Thus, scientists the world-over, are finding it extremely difficult to find a drug that will act only on the invading virus. But Jenner's discovery about the capacity of our body to produce antibodies to fight the disease-causing microbes, is being used as the main plank of defence against many devastating epidemics, especially, of viral origin, like smallpox and poliomyelitis. But this is known as prophylactic or preventive measure and not therapy. But with the increasing knowledge of the fine structure and composition of viruses and with the fast-advancing knowledge in other branches of science, we might be able to design or discover before long, a drug to eliminate selectively virus infections from our body; one can see a ray of hope in this direction.

One thing is clear: Man can never sit and gloat over his discoveries and say, "We have discovered drugs against all the known infectious diseases and we have conquered our enemies." By all accounts the fight against the microbes appears to be a ceaseless battle.

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